CORRELATION BETWEEN INTERNAL SURFACE AND TRANSPIRATION RATE IN MESOMORPHIC AND XEROMORPHIC LEAVES GROWN UNDER ARTIFICIAL LIGHT

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Introduction

The Dalton equation (16), $V = K(F-f)\frac{760}{P}S$, shows the importance of the area of the water surface (S) in the rate of evaporation (V). TURRELL (21), by means of the internal-external surface ratio (R), showed that the internal surface of leaves from which water evaporates in transpiration is several times larger than the external. If leaf transpiration obeys the same physical laws as evaporation, it is evident from the equation that a leaf with a large internal surface would have a higher transpiration rate under the same conditions than one with a small internal surface, when transpiration rate is based on unit external leaf surface.

The internal surfaces of certain leaves measured by the writer were shown to correlate positively with transpiration rates found by other investigators (20, 21). Since these comparisons and the a priori analyses of the evaporation formula are in general agreement, the investigation reported here was undertaken to determine whether such agreement could be verified by experiment.

Material and methods

Plants of oleander (*Nerium oleander* L.) were selected as a type having thick, xeromorphic leaves and plants of periwinkle (*Vinca rosea* L.) as a type having thin, mesomorphic leaves. In addition to having a normally xeromorphic and a normally mesomorphic type, it seemed possible that a certain degree of xeromorphy could be induced in the mesomorphic and xeromorphic types by growing sets of each under two different light intensities, since it has been shown that leaves exposed to intense sunlight have a greater leaf thickness, palisade thickness, etc., than leaves grown in the shade (3, 12, 8, 10, 14, 17, 5, 6, 9, and others).

Successful growth of plants when employing artificial light has been reported (1, 11, 13, 15, 7, 2). Because of the constant light intensity, ease in regulation of the light period, and ease with which results might be duplicated, the use of artificial light seemed desirable both for growing the plants and for measuring transpiration rates.

Four medium-sized, rooted cuttings of periwinkle and four small rooted cuttings of oleander plants were transplanted to ½-gallon Ball mason jars containing a layer of gravel in the bottom and rich loam soil above. The plants were grown for 2 weeks in these jars under greenhouse conditions to allow for acclimatization. The screw caps of the jars had been previously drilled with three holes, and through one of these holes the plant stem had been inserted in transplanting and sealed with plasticene. Glass tubes were inserted in the other holes and sealed to the cap with plasticene. One tube reached to the jar bottom and one to just above the soil level. The leaves were stripped from the plants at the conclusion of the 2-week

acclimatization period. Two plants of each species were placed under low artificial light intensity and two of each species under high artificial light, in a room specially outfitted for the experiments.

This basement room, in which the plants developed a second complement of foliage and in which the transpiration experiments were conducted, was $9\frac{1}{2} \times 12\frac{3}{4} \times 8\frac{1}{2}$ feet. It was coated with white cement and was divided into two sections by a wall-board screen. Two hyperbolic galvanized-iron reflectors 18 inches wide, 52 inches long, and 18 inches deep were suspended—one on either side of the screen. Light of high intensity was supplied by three 500-watt Mazda incandescent lamps placed in one of these reflectors and of low intensity by three 200-watt lamps in the other reflector. A wall exhaust fan moved the 981 cubic feet of air in the room at the rate of 600 cubic feet per minute, preventing excessive heating.[1] Despite outdoor summer temperatures as high as 114° F. in the shade, the temperature in the room never exceeded 93° and averaged 87.7° F., according to thermograph records. The relative humidity, which was not controlled, ranged from 46 to 84%. The barometric pressure was steady at 29.5 inches.

When the plants were first placed under the 500-watt lamps, the maximum intensity measured with a Macbeth illuminometer at the table top on which the plants rested was 300.7 foot-candles ("high intensity") and under the 200-watt lamps was 85.9 foot-candles ("low intensity"). Twenty-eight inches above the table top the high intensity (500-watt) measured 964.5 foot-candles, while under the low intensity (200-watt) it measured 300.4 foot-candles. During the transpiration experiment the intensity averaged 175.0 foot-candles on the table top under the 500-watt lamps and 76.8 foot-candles under the 200-watt lamps. These ranges are far below those of summer noon sun.

During the 60 days in which the plants were allowed to develop new foliage, they were exposed to 12-hour photoperiods per day under the artificial light. All the plants made vigorous growth, and the periwinkles under the high intensity blossomed. At this time it was assumed that the plants had developed some mature foliage and that sufficient differentiation had taken place in the structure of the leaves to affect the transpiration rates.

The plants were then well watered, and the glass watering tubes through the jar cap—which had remained uncorked during the growth period—were corked. The plant potometers were weighed on a double-beam agate-bearing balance having a capacity of 5000 gm.[2] Weighings were then made each morning and evening to determine the water lost by transpiration. This amount was replaced after each light period.

Three experiments were conducted. In the first the transpiration of both periwinkle and oleander plants was measured under the same light intensity in which they were grown. Two 250-cc. beakers, filled to within $\frac{1}{2}$ inch of the brim with distilled water, were placed—one in each intensity—and the water loss by evaporation measured for the same period as for transpiration. Using the

[1] About 3% of radiation of the Mazda lamp is in the visible (19).

[2] Under a load of 2200 gm. the sensitivity of the balance was 0.48 gm./division, while under a load of 3800 gm. it was 1.0. The heaviest potometer weighed about 3600 gm., and the average transpiration loss was 100 gm. between two subsequent weighings. Thus transpiration loss could be measured with an error of not greater than ± 1.0%.

difference in the amount of water evaporated from the beakers as a basis, a correction factor was applied to the transpiration rate of the plants to compensate for the water loss due to the greater radiation in the high intensity. Transpiration and evaporation losses were measured over approximately a 3-day period.

In the second experiment periwinkle and oleander plants with foliage grown under high intensity were placed in low intensity together with similar plants with foliage grown in low intensity. Transpiration and evaporation losses were measured over approximately a 3-day period.

The first and second experiments were conducted in the order given and prior to the third experiment, in order that as little change as possible might be induced in the structure of the immature leaves. For the third experiment, periwinkle and oleander plants with foliage grown in high intensity and with foliage grown in low intensity were all placed under high intensity. Transpiration and evaporation were measured over approximately a 3-day period.

On the conclusion of the transpiration experiments, sections of tissue 0.25 sq. cm. were cut from the center of the blades of an upper and lower leaf of each of the plants and killed in formalin-acetic acid-alcohol solution. From these sections permanent microscope slides were prepared, internal surface measurements made according to the method described by TURRELL (21), and the ratio (R) of the internal surface per unit external surface was determined.

Immediately after selection of the sections for the internal surface measurements, the leaves were stripped from each plant and blueprints made of the leaves with a Daylight carbon-arc lamp. The areas of the leaves were subsequently measured from the blueprints with a planimeter.

The results of the three experiments are not mutually comparable, since the transpiration data have not been reduced to the same number of hours for each of the experiments although the same plants have been used in each.

Results

NATURAL AND INDUCED XEROMORPHY.—The foliage grown in high intensity was more xeromorphic in both periwinkle and oleander than that grown in low intensity (table 1). The foliage of oleander was more xeromorphic than that of periwinkle, regardless of the intensity of light in which it was grown (table 1).

The differences of leaf and tissue thicknesses of the leaves grown in high and in low intensity are shown in table 2. Comparison of the ratios for tissue and leaf thicknesses in both plants shows the relative xeromorphy induced. Table 2 indicates that high intensity induced a relatively greater xeromorphy in periwinkle than in oleander leaves—1.11 times, judging on the basis of leaf thickness, and 3.00 times, judging on the basis of palisade thickness. The effect of high intensity, however, was relatively less (0.73 times) on the thickness of the upper epidermis of the periwinkle than on that of the oleander leaves.

The greater induced xeromorphy in the foliage of the plants grown under high intensity and the greater natural xeromorphy of the oleander plants are reflected in the internal-external surface ratios (R). An average of these ratios for upper and lower leaves of each plant is shown in table 3a. Leaves of both species grown in high intensity had higher internal-external surface ratios than when grown in low intensity. (One-

hundred-sixty determinations of the effect of light intensity on (R) were made for periwinkle, and also for oleander leaves, by a modification of a previous method (23).)

STOMATAL SIZE AND INTERSTOMATAL DISTANCES.—Leaves of periwinkle had stomata more or less regularly distributed on the upper and lower surfaces. The stomata averaged 22.2 μ in length and 14.1 μ in width—including the guard cells. The stomatal pore length averaged 11.4 μ and the width 2.3 μ. In large leaves the interstomatal-pore distance averaged 70.0 μ and in small leaves 61.2 μ. Leaves of oleander had no stoma-

TABLE 1

CHARACTERISTICS OF LEAVES OF PLANTS GROWN UNDER DIFFERENT LIGHT INTENSITIES (THREE LAMPS USED)

Plant	Lamp wattage	Light intensity	Leaf thickness (μ)	Palisade depth (μ)	Thickness of upper epidermis (μ)	Thickness of upper cuticle (μ)
Periwinkle 1, 2	500	High	130	37	21	<1.0
Periwinkle 3, 4	200	Low	108	25	15	<1.0
Oleander 1, 2	500	High	239	64	48	3.5
Oleander 3, 4	200	Low	203	55	31	1.8

TABLE 2

RELATIVE XEROMORPHY DEVELOPED IN PLANTS BY HIGH LIGHT INTENSITY

Plant	Leaf thickness (high light − low light)	Palisade thickness (high light − low light)	Upper epidermal thickness (high light − low light)
Periwinkle	22μ	12μ	6μ
Oleander	36	9	17

	$\frac{\text{Diff. in leaf thickness}}{\text{Leaf thickness}}$	$\frac{\text{Diff. in palisade thickness}}{\text{Palisade thickness}}$	$\frac{\text{Diff. in epidermal thickness}}{\text{Epidermal thickness}}$
Periwinkle	0.20	0.48	0.40
Oleander	0.18	0.16	0.55

	$\frac{\text{Diff. in leaf thickness/leaf thickness}}{\text{Diff. in leaf thickness/leaf thickness}}$	$\frac{\text{Diff. in palisade thickness/palisade thickness}}{\text{Diff. in palisade thickness/palisade thickness}}$	$\frac{\text{Diff. in epidermal thickness/epidermal thickness}}{\text{Diff. in epidermal thickness/epidermal thickness}}$
$\frac{\text{Periwinkle}}{\text{Oleander}}$	1.11	3.00	0.73

ta on the upper leaf surface, while on the lower surface they were crowded together in pits sunk in the lower epidermis, and covered with hairs. The stomata averaged 21.8 μ in length and 15.4 μ in width—including the guard cells. The stomatal pore length averaged 8.0 μ and the width 2.6 μ. The interstomatal-pore distance was 29.3 μ and the interstomatal-pit distance was 113.0 μ.

TABLE 3a

AVERAGE INTERNAL-EXTERNAL SURFACE RATIOS (R) FOR UPPER AND LOWER LEAVES GROWN UNDER DIFFERENT LIGHT INTENSITIES

Grown under high intensity		Grown under low intensity	
Plant	(R)	Plant	(R)
Periwinkle 1	8.86	Periwinkle 3	8.34
Periwinkle 2	8.59	Periwinkle 4	6.57
Oleander 1	14.90	Oleander 3	13.35
Oleander 2	18.85	Oleander 4	15.10

Experiment I: Transpiration measured simultaneously in low and in high light intensity

A. The transpiration losses in high intensity of two periwinkle and two oleander plants, with foliage grown under high intensity, are shown in table 4. These losses, reduced to grams of water transpired per cm.^{2} of leaf surface, are shown in table 5. If the length of the experimental period is considered as a unit time, the values shown are transpiration rates.

The transpiration rate of oleander was greater than that of periwinkle when measured in high intensity (table 5). The (R) was greater in the oleander plants having foliage grown in high intensity than similarly grown foliage of periwinkle (table 3a). The calculated coefficient of correlation (r) between the transpiration rate and (R) is +0.787 (table 6). This value is high and positive but not sufficiently high for 2 degrees of freedom to be significant.

B. Simultaneously, and for the same length of time as in A, transpiration losses in low intensity of the two periwinkle and two oleander plants, having foliage grown in low intensity, were measured (table 4). The transpiration rates were greater in oleander than in periwinkle (table 5). The (R's) were also greater for oleander (table 3a). The coefficient of correlation between the transpiration rate and (R) is +0.744 (table 6). This value is high and positive but not sufficiently high for 2 degrees of freedom to be significant.

TABLE 3b

SUMMARY OF ANALYSIS OF VARIANCE OF (R)

Source of variation	Degrees of freedom			Sum of squares			Mean square		
	Periwinkle vs. oleander	Periwinkle high vs. low light	Oleander high vs. low light	Periwinkle vs. oleander	Periwinkle high vs. low light	Oleander high vs. low light	Periwinkle vs. oleander	Periwinkle high vs. low light	Oleander high vs. low light
Total	7	159	159	130.87	386.99	1545.75	18.68	.....	.....
Between	1	1	1	111.30	81.76	302.37	111.30*	81.76*	302.37*
Within	6	158	158	19.59	305.23	1243.38	3.26	1.93	7.87

* Highly significant.

C. The total loss of water from the beakers as evaporation in high and in low intensity, measured simultaneously and for the same length of time as the transpiration of the plants in A and B, was 106 and 88 gm., respectively (table 7). Reducing these values to evaporation per unit area of free water surface gives 1.70 and 1.42 gm./cm.²/period for evaporation in high and in low intensity, respectively. The difference in the rate of evaporation (19.7%) in the two beakers is due primarily to the difference in intensity of the radiation incident upon the water in them. The transpiration rate values of periwinkle plants measured in high intensity were reduced 19.7% to equalize the effects of light intensity.

After making these equalizations, the correlation coefficient between (R) and the transpiration rate of periwinkle plants with foliage grown in high intensity and the transpiration measured in high and of such plants with foliage grown in low intensity and the transpiration measured in low was +0.996 (table 6). This value is high, positive, and highly significant.

D. The transpiration rate values of oleander plants measured in high in-

TABLE 4

EFFECT OF LIGHT INTENSITY ON TOTAL TRANSPIRATION OF PLANTS GROWN UNDER HIGH AND LOW INTENSITIES*

Plant	Total upper and lower leaf surface (cm.²)	Transpiration			
		Experiment I		Experiment II	Experiment III
		Low intensity (gm.)	High intensity (gm.)	Low intensity (gm.)	High intensity (gm.)
	Grown under high intensity				
Periwinkle					
No. 1	2720	.....	549	639	531
No. 2	1890	.....	334	428	300
Oleander					
No. 1	551	.....	261	430	415
No. 2	414	.....	150	297	268
	Grown under low intensity				
Periwinkle					
No. 3	1920	241	.....	459	381
No. 4	616	55	.....	91	51
Oleander					
No. 3	547	125	.....	454	409
No. 4	538	82	.....	437	327

* Duration of experiments I, II, and III is not the same, thus the transpiration losses per experimental period are not mutually comparable.

tensity were reduced 19.7% to equalize the effects of radiation on transpiration losses. Despite this reduction, the rates for oleander with foliage grown in high and transpiration measured in high and the transpiration when grown in low and transpiration measured in low are correlated with internal surface only to a moderate degree. The correlation coefficient of +0.428 is not statistically significant.

E. After applying the correction factor, if all eight plants in this experiment are considered together in calculating the correlation coefficient between transpiration rate and (R), the value of +0.852 is high and highly significant.

Experiment II: Transpiration measured in low light intensity

Plants of both species grown in high intensity were placed in low together with both types grown in low, and transpiration of all plants was measured. The total transpiration losses are shown in table 4, and the losses per cm.²/period are given in table 5.

TABLE 5

Effect of light intensity on transpiration rates of plants grown under high and low intensities*

Plant	Transpiration			
	Experiment I		Experiment II	Experiment III
	Low intensity (gm./cm.²/period)	High intensity (gm./cm.²/period)	Low intensity (gm./cm.²/period)	High intensity (gm./cm.²/period)
	Grown under high intensity			
Periwinkle				
No. 1	.....	0.202	0.235	0.195
No. 2	.....	0.177	0.226	0.159
Oleander				
No. 1	.....	0.474	0.781	0.754
No. 2	.....	0.362	0.718	0.647
	Grown under low intensity			
Periwinkle				
No. 3	0.125	.....	0.239	0.198
No. 4	0.090	.....	0.148	0.083
Oleander				
No. 3	0.228	.....	0.829	0.748
No. 4	0.152	.....	0.814	0.608

* Duration of experiments I, II, and III is not the same, thus the transpiration rates per experimental period are not mutually comparable.

A. A high correlation existed between (R) and transpiration rate in periwinkle. The (r) was +0.941, but it is of doubtful significance as it is below the 5% level although above the 10% level.

B. In the oleander plants, (r) was

TABLE 6

CORRELATION COEFFICIENTS FOR RELATION OF TOTAL TRANSPIRATION OF PLANTS GROWN UNDER DIFFERENT LIGHT INTENSITIES AND MEASURED UNDER SAME OR DIFFERENT INTENSITIES, AND INTERNAL-EXTERNAL SURFACE RATIO (R)

EXP. NO. AND COMPARISON DESIGNATION	PLANT		CORRELATION COEFFICIENT OF TRANSPIRATION			DEGREES OF FREEDOM	SIGNIFICANCE†
	Grown under high intensity	Grown under low intensity	High intensity	Low intensity	Intensity in which grown		
I. A	Periwinkle\ Oleander /		+0.787			2	Ns
I. B		Periwinkle\ Oleander /		+0.744		2	Ns
I. C	Periwinkle*	Periwinkle			+0.996	2	Hs
I. D	Oleander*	Oleander			+0.428	2	Ns
I. E	Periwinkle* Oleander /	Periwinkle* Oleander /			+0.852	6	Hs
II. A	Periwinkle	Periwinkle		+0.941		2	G
II. B	Oleander	Oleander		−0.953		2	S
II. C	Periwinkle\ Oleander /			+0.915		2	G
II. D		Periwinkle\ Oleander /		+0.988		2	S
II. E	Periwinkle\ Oleander /	Periwinkle\ Oleander /		+0.893		6	Hs
III. A	Periwinkle	Periwinkle	+0.940			2	G
III. B	Oleander	Oleander	−0.538			2	Ns
III. C	Periwinkle\ Oleander /		+0.890			2	Ns
III. D		Periwinkle\ Oleander /	+0.946			2	G
III. E	Periwinkle\ Oleander /	Periwinkle\ Oleander /	+0.890			6	Hs

* Corrected for evaporation caused by difference in radiation intensity.

† Ns = not significant; G = above 10% level; S = above 5% level (significant); Hs = above 1% level (highly significant).

TABLE 7

EFFECT OF DIFFERENT LIGHT INTENSITIES ON EVAPORATION OF WATER IN CONTAINERS (FREE WATER SURFACE)

FREE WATER SURFACE	SURFACE AREA (CM.²)	EVAPORATION							
		Experiment I				Experiment II		Experiment III	
		Low intensity		High intensity		Low intensity		High intensity	
		gm./ period	gm./cm.²/ period	gm./ period	gm./cm.²/ period	gm./ period	gm./cm.²/ period	gm./ period	gm./cm.²/ period
No. 1	62.1	.....	.....	106	1.70	46	0.743	100	1.61
No. 2	62.1	88	1.42	.....	.....	47	0.759	100	1.61

−0.953, which is high, negative, and significant (table 6).

C. When both the periwinkle and oleander plants grown in high intensity are considered, the correlation between (R) and transpiration rate is high and positive (r = +0.915), but it is only significant at the 10% level (table 6).

D. When both the periwinkle and oleander plants grown in low intensity are considered, the correlation coefficient between (R) and transpiration rate is high, positive (r = +0.988), and significant (table 6).

E. The best test of the relation between (R) and transpiration rate results from consideration of all the plants together, regardless of the light intensity in which they were grown. In this case the (r) is +0.893, which is high, positive, and highly significant—above the 1% level (table 6).

Experiment III: Transpiration measured under high light intensity

On completion of experiment II, all the plants (both periwinkle and oleander grown in high and low intensity) were placed in high and their transpiration measured. Total transpiration losses are shown in table 4 and losses per cm.^{2}/period in table 5.

A. A high correlation existed between (R) and transpiration rate in periwinkle. The (r) was +0.940, but it is of doubtful significance as it is below the 5% level although above the 10% (table 6).

B. In oleander the (r) was −0.538, which is moderate, negative, and not significant (table 6).

C. When both the periwinkle and oleander plants grown in high intensity are considered, the correlation between (R) and transpiration rate is high and positive (r = +0.890), but it is just below the point of significance at the 10% level (table 6).

D. When both species grown in low intensity are considered, the correlation coefficient between (R) and transpiration rate is high, positive (r = +0.946), and of doubtful significance, as it is just below the 5% level and above the 10% (table 6).

E. As in experiments I and II, the best test of the relation between (R) and transpiration rate results from consideration of all the plants, regardless of the light intensity in which they were grown. In this case the correlation coefficient is +0.890, which is high, positive, and highly significant (table 6).

Relation of stem transpiration to total transpiration

To determine the maximum error introduced by treating total transpiration as leaf transpiration, the defoliated stems of all the plants used in these experiments were placed in high intensity and illuminated continuously for 11 days. A fan was turned directly on the plants to induce as great water loss as possible. Under these conditions the average transpiration per day was 1.34 gm. per stem, as compared with approximately 100 gm. per day per normal plant. Thus the maximum error in using total transpiration as leaf transpiration is approximately 1.34%.

Discussion

The experiments as conducted and the conclusions drawn from them are based on several assumptions: (*a*) that the roots of the plants were in an active and healthy growing condition, so that they were able to absorb water readily; (*b*) that the soil contained ample water, easily to supply all needs of the plants; and (*c*) that the evaporating conditions

for the tops were not so great that the rate of water absorption by the roots was a limiting factor. It was concluded that these assumptions were reasonably correct, for the root systems were well developed and no dead roots were found at the conclusion of the experiment; the plants were well watered during the experiments and the soil in the potometers moist at their conclusion; and no wilting of the leaves was observed at any time.

The effect of stomatal control over transpiration was not measured, but the effects so far as these experiments were concerned were minimized as much as possible by selecting two genera with fundamentally different stomatal distributions. Also, the use of plants grown in different light intensities but with transpiration measured under the same intensity tends to minimize the effect of stomata, for similar stomatal distributions permit water vapor diffusion from different-sized areas of internal surface.

Using SAYRE'S modification (**18**) of BROWN and ESCOMBE'S law (**4**), it was readily calculated that the stomata in the leaves of periwinkle were 2.5 times too close together to secure maximum diffusion in still air; in the stomatal pits of oleander it was 5.74 times. In the latter, when the pits were considered as a unit, the stomatal groups were still 1.49 times too close for maximum diffusion.

Diffusion is proportional to pore area in moving air (**4**). In the periwinkle leaves the area of a stomatal pore averaged 81.3 μ^2 and in the oleander 65.5 μ^2. The stomatal pits, located on only one side of the oleander leaves, contained eight to ten stomata per pit. The stomata in the periwinkle leaves were located on both leaf surfaces, but it was estimated that the oleander leaves would suffer less interference with diffusion in moving air. They have approximately 184,000 μ^2 of pore area per mm.^{2} of leaf area, while periwinkles have approximately 110,000 μ^2 of pore area. These figures suggest that a positive correlation may exist between stomatal pore area per mm.^{2}, internal leaf surface, and transpiration rate.

Stem transpiration—although appreciable—was too small to influence significantly the results of leaf transpiration. The error in the measurement of transpiration rate likewise was small. The internal surface measurements were extremely accurate for the sample taken. However, the small number of samples which could be analyzed undoubtedly made the sampling error large. Two other factors—the horizontal and the vertical light distribution as described by the inverse square law—had an effect on the morphological structure of the leaves. This has been discussed (**22**), and it may suffice here to say that artificial light is excellent for many types of experimental work with plants, but it does not provide a good light source for an experiment of this kind (**22**). Three to four per cent of the radiation of a Mazda lamp is in the visible range (**19**), and this quality of the lamp may also have influenced the structure and behavior of the plants to some extent.

My interpretation of the significant correlation between the transpiration rates and internal-external surface ratios of periwinkle leaves developed under the two intensities, when measured under two intensities in the first experiment, is that a very significant degree of xeromorphy had been induced by the high intensity under 500-watt lamps (table 2). Other correlations, in which four oleander plants were used alone or two oleander with two periwinkle plants, were not significant in the first experiment because only a small (although signifi-

cant) degree of xeromorphy was developed in the oleander leaves (table 2). This fact, together with the limited number of degrees of freedom available, was sufficient to reduce the correlation coefficients to nonsignificance, although they were positive. In the correlations based on all the plants the natural xeromorphy and high transpiration rates of the oleander leaves contributed considerable weight, and with the larger number of degrees of freedom gained, gave correlation coefficients which were high, positive, and highly significant (table 6).

In experiments II and III the correlation coefficients between transpiration rate and (R) are high and significant at the 5% level, 10% level, or are nearly significant when four plants are compared (except for oleander plants alone). The improvement in the correlations in the latter experiments over the first is probably dependent on the fact that all the plants were under the same light intensity, and that the limitations of a correction factor for different intensities did not enter into the calculations. When all the plants were used in calculating a correlation coefficient between (R) and transpiration rate, (r) was high, positive, and highly significant.

No investigation was made of the causes underlying the high negative and significant correlation in experiment II, where the transpiration rates of oleander plants were measured under low intensity, and there seems no complete explanation for it. Likewise, in experiment III the same comparison under high intensity gives a lower but nonsignificant negative correlation coefficient. Several factors are probably involved, however, among which are the effect of light intensity and stomatal closure under low intensity in the leaves which had been grown under high intensity.

In experiment II, when transpiration rate of periwinkle plants is compared with evaporation from a free water surface, the transpiration/evaporation ratio was 0.28, and in oleander it was 1.05. In experiment III the ratios were 0.10 and 0.43 for periwinkle and oleander, respectively. While the absolute values for transpiration are not comparable in the two experiments, the relative rates of transpiration to evaporation are. Calculation shows that the periwinkle plants reduced their relative transpiration/evaporation ratio under high intensity 64.3% and the oleander plants 59.1%. Both experiments indicate the efficiency of the leaf in conserving water, for the internal transpiring surface of the periwinkle leaves per cm.^{2} of leaf area averaged 16.18 times the unit free water surface (1 cm.^{2}), and the internal transpiring surface of oleander per cm.^{2} of leaf area averaged 31.10 times. These calculations also suggest that the more drastic the evaporating conditions, at least over a certain range, the more efficient the leaf mechanism becomes in conserving water as compared with a free water surface.

The actual efficiency of the leaf can be more readily appreciated when it is recalled that the internal transpiring leaf surface in periwinkle averaged 8.1 times the external surface, and in oleander, 15.6. For example, in experiment III the actual ratio of water evaporated (transpired) from the internal leaf surface per cm.^{2}/period to the evaporation of water from a free water surface per cm.^{2}/period is 0.012 for periwinkle and 0.027 for oleander. These calculations show that the transpiration is much less per unit leaf internal surface than evaporation from a free water surface. They also show that the oleander leaves transpired on the average more than twice as much per unit area of internal surface as the peri-

winkle, indicating the existence of leaf differences in the two genera other than area of internal leaf surface. These differences may be in part morphological, but most likely they are physico-chemical (for example, osmotic pressure).

The experiments indicate that the size of the area of internal exposed surface per unit external leaf surface is strongly correlated with transpiration rates per unit area, as might be surmised from examination of the Dalton equation (16) or other equations for the physical evaporation of water. Other factors also are shown to be partially responsible for the high correlation of internal leaf surface and transpiration rate.

Summary

1. Plants of periwinkle and oleander were grown under two ranges of intensity of artificial light. The low intensity range was 86–300 foot-candles and the high intensity range was 301–965 foot-candles. Both the mesomorphic leaves of periwinkle and the xeromorphic leaves of oleander developed a significant degree of xeromorphy under high intensity, when judged on the basis of the relative increase in palisade thickness.

2. The xeromorphic leaves of oleander had significantly greater internal-external surface ratios than the mesomorphic leaves of periwinkle. The leaves of both species grown under high intensity had significantly larger internal-external surface ratios than the leaves of those grown under low intensity.

3. Transpiration rates of both species were measured by weighing in potometers. When all the plants were treated statistically as a group, correlations between the internal-external surface ratios and the transpiration rates were high and highly significant ($r = +0.85$ to $+0.89$). These correlations obtained, whether transpiration was measured under low (minimum 77 foot-candles), under high (minimum 175), or under the light intensity in which the plants were grown, when in the latter instance correction was made for radiation. Individual comparisons, however, were often not significant.

4. The xeromorphic leaves of oleander had greater transpiration rates than the mesomorphic leaves of periwinkle per unit external leaf surface, as predicted from evaporation equations for free water surfaces; but computations of transpiration per unit internal leaf surface indicated other factors were also responsible for the greater transpiration rate in xeromorphic leaves.

The writer is indebted to Professor W. F. LOEHWING of the University of Iowa for supplying the material and facilities and to Professor R. B. WYLIE, in whose laboratory the internal leaf surface measurements were made.

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EFFECTS OF VARIATION IN NUTRIENT SOLUTION ON GROWTH OF SUNFLOWER PLANT

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 559

SCOTT V. EATON

Introduction

For some time the writer has been studying the effects of sulphur deficiency on the growth of plants, and a summary paper has recently been published (4). In much of this work, nutrient solution IR2S4 of the LIVINGSTON triangle (11) was used, since preliminary work indicated that it was best for growth of the sunflower. Others have used this solution (13). In the sulphur-deficiency work on the mustard (4), this solution was diluted somewhat, since growing the plant at various dilutions indicated that at full strength it was too concentrated for the mustard. In preparation for work with the sunflower on a different phase of mineral nutrition, therefore, it was decided to compare the effects on its growth of IR2S4 with dilutions of this solution and with solutions of other investigators (9, 18).

METHODS.—The plants were grown in the spring and summer of 1943 by essentially the same methods as previously used (3). The seeds were removed from the achenes, selected for uniformity, and planted—twelve per pot—in pure quartz sand in glazed 2-gallon pots, which were provided with a hole in the bottom for

drainage. Ten days to 2 weeks after planting, the plants were thinned to three uniform ones per pot. There were two crops; the growth period of the first was May 11 to June 17 (38 days); of the second, July 1 to August 5 (36 days). There were 359 plants in each crop, divided into five series of sixty plants and one series of fifty-nine. A different nutrient solution was used to water the plants of each of the six series.

Table 1 gives the molar concentrations of the salts of the six solutions which were compared, table 2 gives the parts per million of each element and the total for the salts, and table 3 gives the pH of the original solutions and of the drip obtained by flushing with distilled water or with the solutions.[1] The two flushings with nutrient solution were on different days. The pH was determined by means of a Coleman electrometer equipped with a glass electrode. The osmotic pressure of solution 3 (IR2S4) was 1 atmosphere (11), and the approximate osmotic pressure of solutions 1, 2, 4, 5, and 6 was 0.25, 0.5, 0.9, 0.7, and 0.5 atm., respectively. A solution of ferric citrate at a concentration of 1 p.p.m. of iron was sprinkled on the sand once a week, and copper chloride and zinc chloride at concentrations of 0.02 and 0.05 p.p.m., respectively, of copper and zinc were added every 2 weeks.

Enough was added to each pot daily until considerable solution drained out. The sand was flushed once a week, fresh nutrient solution being added immediately afterward.

Data

The weights of the plants produced by the six solutions are given in tables 4 and 5 and figure 1. In each crop the plants produced by solution 5 were the largest, followed in order by solutions 4, 3, 2, 6, and 1. The order is the same for each of the plant parts in the first crop, but in the second crop the roots of solution 5 weighed slightly less than those of solution 4, and the roots and leaves of solution 6 more than those of solution 2, although these differences are not great enough to be significant. Tables 4 and 5 also give the top-root ratios. These are consistently smaller for the second crop.

The data of tables 4 and 5 and figure 1 show that the plants grown with solution 3 rank third in size. In tables 6 and 7 the comparison is on a percentage ba-

TABLE 1

MOLAR CONCENTRATION OF SALTS OF NUTRIENT SOLUTION*

Solution	Salts				
	$Ca(NO_3)_2$	KH_2PO_4	$MgSO_4$	KNO_3	$(NH_4)_2SO_4$
1. IR2S4† (¼ conc.)	0.00225	0.00112	0.00112	.....	.....
2. IR2S4 (½ conc.)	0.00450	0.00225	0.00225	.....	.....
3. IR2S4 (full conc.)	0.00900	0.00450	0.00450	.....	.....
4. IR2S4 (low N)	0.00600	0.00450	0.00450	.....	.....
5. Hoagland and Arnon	0.00500	0.00100	0.00200	0.0050	.....
6. Shive and Robbins	0.00450	0.00230	0.00230	.....	0.00070

* Each solution also contained boric acid and manganese chloride at concentrations of 0.5 p.p.m. of each of the elements boron and manganese. A solution of ferric citrate at a concentration of 1 p.p.m. of iron was sprinkled on the sand once a week, and copper chloride and zinc chloride at concentrations of 0.02 and 0.05 p.p.m. of copper and zinc were added every 2 weeks.

† A solution of the LIVINGSTON triangle (11).

[1] Solutions are referred to hereafter mainly by number.

sis. The plants of solution 5 were more than 17% larger than those grown with solution 3, while those of solution 4 were more than 5% larger in the first crop and 9% in the second. The plants of the other solutions were smaller than those of solution 3—very much smaller in the case of solution 1.

about 6 miles from the greenhouse were obtained. While light and temperature (especially temperature) conditions at the airport and in the greenhouse would naturally differ, a comparison of the data for the two growth periods is not without significance in explaining the differences in the size of the plants of the two crops.

TABLE 2

ELEMENTS OF NUTRIENT SOLUTIONS (P.P.M.)

Solution	Ca	N	K	P	Mg	S	Mn	Bo	Total for salts
1. IR2S4 (¼ conc.)	90.2	63.0	44.0	34.9	27.4	36.1	0.5	0.5	661.7
2. IR2S4 (½ conc.)	180.4	126.1	88.0	69.7	54.7	72.1	0.5	0.5	1319.5
3. IR2S4 (full conc.)	360.7	252.1	175.9	139.4	109.4	144.3	0.5	0.5	2635.1
4. IR2S4 (low N)	240.5	168.1	175.9	139.4	109.4	144.3	0.5	0.5	2142.8
5. Hoagland and Arnon	200.4	210.1	234.6	31.0	48.6	64.1	0.5	0.5	1706.8
6. Shive and Robbins	180.4	145.7	89.9	71.3	55.9	96.1	0.5	0.5	1424.8

TABLE 3

PH OF ORIGINAL SOLUTIONS AND OF DRIP

Solution	Original solution	Drip by flushing with nutrient solution	Drip by flushing with nutrient solution	Drip by flushing with distilled water	Range of all determinations
1. IR2S4 (¼ conc.)	5.21	6.55–6.92	5.71–6.40	5.37–6.55	5.37–6.92
2. IR2S4 (½ conc.)	4.87	6.53–7.13	6.12–6.32	6.47–6.80	6.12–7.13
3. IR2S4 (full conc.)	4.61	5.57–6.17	5.67–6.30	5.55–5.80	5.55–6.30
4. IR2S4 (low N)	4.66	6.14–6.60	5.79–6.15	5.35–6.07	5.35–6.60
5. Hoagland and Arnon	5.00	6.81–7.82	6.73–7.56	6.71–7.92	6.71–7.92
6. Shive and Robbins	4.81	5.87–6.28	5.72–6.02	5.61–5.96	5.61–6.28

The data of tables 4 and 5 and figure 1 show that the plants of the second crop were considerably larger than those of the first. Table 8 places the comparison on a percentage basis. The effects of the higher temperatures and the greater amount of sunshine of the second growth period are clearly shown.

Continuous records of temperature and sunshine in the greenhouse where the plants were grown were not taken, but data of the U.S. Weather Bureau Station located at the municipal airport

The average daily maximum, minimum, and mean temperatures for the first growth period were 71.9, 54.5, and 63.2, respectively, and for the second 86.0, 65.6, and 75.8. There were only 2 days during the first period when the temperature reached 90° F. or above; there were 13 such days during the second. The sun shone 54.9% of possible shine during the first growth period and 68.8% during the second. These differences in temperature and sunshine are sufficient to account for the larger plants of the second crop.

In the case of each solution, the increased weights of the plants of the second crop were greatest for the roots and least for the leaves, the stems being intermediate. For the entire plant the increase ranged from 54% for solution 1 to 37% for solution 2, the other solutions being between these extremes.

But, as ARNON and HOAGLAND (1) and others have found, suitable and fairly uniform plant growth may be obtained with solutions varying considerably in both total concentration and salt proportions, provided there is present at each stage of growth a sufficient amount of each essential element. The present

TABLE 4

GRAMS PER 60 PLANTS, WET WEIGHT
(GROWTH PERIOD, MAY 11–JUNE 17)

Solution	Stems	Leaves	Tops	Roots	Entire plant	Top-root ratio
1. IR2S4 ($\frac{1}{4}$ conc.)	1878.3	818.4	2696.7	364.0	3060.7	7.409
2. IR2S4 ($\frac{1}{2}$ conc.)	3220.2	1410.2	4630.4	482.5	5112.9	9.597
3. IR2S4 (full conc.)	3466.1	1529.2	4995.3	568.1	5563.4	8.793
4. IR2S4 (low N)	3659.0	1598.2	5257.2	614.6	5871.8	8.554
5. Hoagland and Arnon	4214.8	1677.0	5891.8	633.0	6524.8	9.308
6. Shive and Robbins	2981.4	1265.7	4247.1	394.3	4041.4	10.771

TABLE 5

GRAMS PER 60 PLANTS, WET WEIGHT
(GROWTH PERIOD, JULY 1–AUGUST 5)

Solution	Stems	Leaves	Tops	Roots	Entire plant	Top-root ratio
1. IR2S4 ($\frac{1}{4}$ conc.)	2987.0	1019.5	4006.5	732.3	4738.8	5.471
2. IR2S4 ($\frac{1}{2}$ conc.)	4599.2	1498.5	6097.7	923.5	7021.2	6.603
3. IR2S4 (full conc.)	5174.0	1801.7	6975.7	1045.1	8020.8	6.675
4. IR2S4 (low N)	5727.0	1925.5	7652.5	1095.0	8747.5	6.989
5. Hoagland and Arnon	6389.4	2030.7	8420.1	1039.8	9459.9	8.098
6. Shive and Robbins	4353.0	1561.5	5914.5	924.1	6838.6	6.400

The top-root ratios of the second crop were smaller than those of the first (tables 4, 5). Table 8 shows that this is due to the relatively greater growth of the roots than of the tops during the second growth period.

Discussion

COMPARISON OF THE SIX SOLUTIONS

Much work has been done in an attempt to determine the best concentration and proportion of salts of the nutrient solution for the growth of plants. work supports this conclusion. While the plants grown with the various solutions varied considerably in size, their growth in all series was satisfactory, except those grown with solution 1 (tables 4, 5; fig. 1). This solution had an osmotic pressure of about 0.25 atm. and was evidently too dilute—at least under the conditions of this experiment—to supply sufficient nutrient elements. Tables 6 and 7 compare solution 3 (IR2S4) with the other solutions. The plants of solution 1 were 44.99% smaller than those of solution 3

in the first crop and 40.92% smaller in the second. The plants of the other four solutions were much closer to the size of the plants of solution 3, ranging from 17.94% larger in the case of solution 5 of the second crop to 16.57% smaller in solution 6 of the first. The plants of the other two solutions were between these extremes.

While satisfactory plants may be grown with solutions varying considerably in concentration and proportion of salts, this does not mean that consistent and significant differences may not be obtained by rather small variations in the nutrient solutions. As shown by figure 1, the plants of the second crop were considerably larger than those of the first, but the curves are of the same type. The difference between the plants of IR_2S_4 and each of the other solutions is the same in each crop within about 2–4% (tables 6, 7). Although the differences between IR_2S_4 and the other solutions are not large, except in the case of solution 1, they are probably significant. They are consistent in the two crops, and the number of plants of the two harvests of each series total 120 (119 in solutions 1 and 6).

But the results obtained with solutions varying in concentration or proportion of salts vary decidedly, depending on the method of application (**1,18**). If the solution is renewed intermittently or continuously at a low rate, or if the volume of solution is small, a higher concentration will be needed than with rapid continuous renewal. In the latter case the concentration may vary decidedly without materially affecting growth. By the continuous-renewal method, ROBBINS (**17**) secured excellent and uniform growth of tomato plants with solutions ranging in osmotic pressure from 0.44 to 1.70 atm. Even plants grown with a solution of 0.08 atm. approached in weight the plants of the solutions just mentioned, when the renewal was rapid. Continuous renewal also greatly diminishes changes in the pH and ionic proportions, owing to differential absorption of ions. In intermittent renewal these changes may be great enough decidedly to affect the growth of plants.

Different phyla of plants seem to vary as to the optimal concentration of the nutrient solution. VOTH (**21**) found that *Marchantia polymorpha* made the greatest vegetative growth on solutions ranging in osmotic concentration from 0.18 to 0.21 atm. In the present work the plants grown with solution 5 were the largest. This had an osmotic pressure of approximately 0.72 atm. In work with the mustard (**4**) it was found that when the solution was added daily to the sand, a nutrient solution of approximately 0.5 atm. resulted in larger plants than one of 1.0 atm. But there was no solution between 0.5 and 1.0 atm. It is possible that the plants would have been larger if a solution somewhat more concentrated than 0.5 atm. had been used. In both the mustard and the present work the nutrient solution was added to the sand once each day. It would seem that with

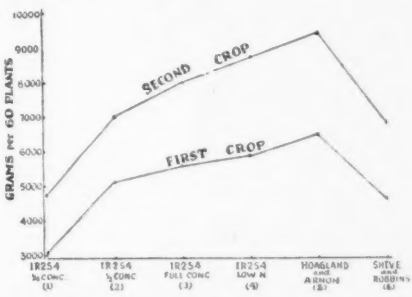

FIG. 1.—Comparison of weights of sunflower plants grown with six nutrient solutions. Growth period of first crop, May 11 to June 17; of the second, July 1 to August 5.

this type of application the optimal concentration for the mustard and sunflower lies between 0.5 and 1.0 atm. However, solution 5 also differs from the others in its salt proportions (table 1), and, as will be shown, this is another possible reason for its superiority.

Concentration alone can hardly account for the relative growth of the plants of solutions 1, 2, and 6 is probably due to the low concentration of their salts. Solutions 4 and 5, with approximate osmotic pressures of 0.90 and 0.72 atm. are probably about optimal in concentration. It would hardly be expected that solution 3 (IR_2S_4, osmotic pressure 1 atm.) would be too concentrated or that the slightly lower concentration of

TABLE 6

COMPARISON OF IR_2S_4 (NO. 3) WITH THE OTHER SOLUTIONS*

(GROWTH PERIOD, JULY 1–AUGUST 5)

Solution	Stems	Leaves	Tops	Roots	Entire plant
1. IR_2S_4 ($\frac{1}{4}$ conc.)	45.81−	46.48−	46.02−	35.92−	44.99−
2. IR_2S_4 ($\frac{1}{2}$ conc.)	7.09−	7.78−	7.30−	15.07−	8.10−
4. IR_2S_4 (low N)	5.57+	4.51+	5.24+	8.19+	5.54+
5. Hoagland and Arnon	21.6 +	9.67+	17.95+	11.42+	17.28+
6. Shive and Robbins	13.98−	17.23−	14.98−	30.59−	16.57−

* Figures represent percentage increase or decrease in size of plants of other crops compared with IR_2S_4 and are obtained by dividing wet weights of plants of IR_2S_4 into the differences between these weights and those of corresponding plant parts of other solutions. Minus means smaller than IR_2S_4; plus means larger.

TABLE 7

COMPARISON OF IR_2S_4 (NO. 3) WITH THE OTHER SOLUTIONS*

(GROWTH PERIOD, JULY 1–AUGUST 5)

Solution	Stems	Leaves	Tops	Roots	Entire plant
1. IR_2S_4 ($\frac{1}{4}$ conc.)	42.27−	43.41−	42.56−	29.93−	40.92−
2. IR_2S_4 ($\frac{1}{2}$ conc.)	11.11−	16.83−	12.59−	11.64−	12.46−
4. IR_2S_4 (low N)	10.69+	6.87+	9.70+	4.78+	9.06+
5. Hoagland and Arnon	23.49+	12.71+	20.71+	0.51−	17.94+
6. Shive and Robbins	15.87−	13.33−	15.21−	11.58−	14.74−

* See footnote to table 6.

plants in the various solutions. This is especially true of solution 5 (HOAGLAND and ARNON), as just mentioned. However, the other five solutions have the same salt proportions, except that solution 4 is somewhat lower in calcium nitrate and solution 6 contains a small amount of ammonium sulphate. The rather high magnesium and phosphate contents of solutions 3 and 4 may be important in accounting for the smaller plants of these solutions as compared with solution 5. The reduced size of the solution 4 (approximate osmotic pressure 0.9 atm.) would account for the larger plants of the latter solution in comparison with the former. Yet the only difference between these solutions is the somewhat lower calcium nitrate content of solution 4. This would be expected to lower—rather than to increase—growth. As shown by tables 6 and 7, the plants of solution 4 were 5% larger than those of solution 3 in the spring crop and 9% in the summer crop. The higher concentration of the latter may account for these

small differences. Concentrated solutions decrease water absorption and the plants assume a xerophytic aspect, as NIGHTINGALE (**14**), HAYWARD and LONG (**7**), and others have shown. Solution 3 was not concentrated enough to cause xerophytic characters, but it is possible that water absorption may have been lessened sufficiently to account for the slightly decreased growth of the plants of this in comparison with solution 4. In the previous work with the mustard (**4**), it was found that a solution of 1 atm. was too concentrated. As just given, the superiority of solution 4 is greater in the summer than in the spring, and this is to be expected if water absorption is the important factor.

TABLE 8

COMPARISON OF FIRST AND SECOND CROPS*

Solution	Stems	Leaves	Tops	Roots	Entire plant
1. IR2S4 (¼ conc.)	59.03	24.57	48.57	101.18	54.83
2. IR2S4 (½ conc.)	42.82	6.26	31.69	91.40	37.32
3. IR2S4 (full conc.)	49.27	17.82	39.65	83.96	44.17
4. IR2S4 (low N)	56.52	20.48	45.56	78.16	48.97
5. Hoagland and Arnon	51.59	21.09	42.91	64.27	44.98
6. Shive and Robbins	46.01	23.37	39.26	134.36	47.34

* Figures represent percentage increase in weights of plants of second crop compared with first and are obtained by dividing wet weights of plants of first crop into the differences between these weights and those of corresponding plant parts of second crop.

POSSIBLE REASONS FOR THE SUPERIORITY OF SOLUTION 5

The plants of solution 5 were larger than those of any other solution. While the concentration of this solution is probably about optimal, it also differs from solutions 4 and 3 (ranking second and third, respectively, in salt proportions) and these differences may be important. (Solutions 1, 2, and 6 are not included in the comparison, for—as already mentioned—their low concentration probably accounts for the smaller plants of these solutions as compared with the other three.) Solution 5 is much lower in phosphorus, magnesium, and sulphur than are solutions 4 and 3 (table 2). While the low sulphur content is probably not significant, the small amount of magnesium and phosphorus may be. Also, in contact with the roots of the plant, solution 5 attains a much higher pH than is true of the other solutions. However, this high pH would be expected to inhibit rather than to increase the growth of the plants.

LOW PHOSPHATE CONTENT

There is considerable work indicating that a plant growing in either soil or a nutrient solution can obtain all the phosphorus it can use from solutions of very low phosphate content. In solution cultures, TEAKLE (**19**) found that 1 p.p.m. of phosphate produced practically as large plants as larger amounts. HOAGLAND and MARTIN (**8**) found 9 p.p.m. of phosphate optimal in one experiment, but in the second experiment the plants grown with 1.1 p.p.m. were almost as large as when higher concentrations were used. Using large volumes of culture solution and renewing the phosphate content once or twice a day, PARKER (**15**) secured maximum growth of corn and soybean with 0.5 p.p.m. of phosphate. The results indicated that if the phosphate content could have been

maintained constant, a still lower concentration would have sufficed for maximum growth. In a companion piece of work, PIERRE and PARKER (16) found that the inorganic phosphate content of the displaced solution of several soils giving no response to phosphorus fertilization was only 0.03–0.04 p.p.m. (The organic phosphorus of the displaced solution was unavailable to plants.) This is considerably lower than that found optimal by PARKER for growth of corn and soybean and indicates that the displaced solution does not represent completely the solution from which the phosphorus is absorbed.

Since maximum growth of plants may occur with nutrient solutions containing phosphorus in very low concentrations, it would be expected that care should be used to avoid toxic effects due to a too high concentration of this element. MOORE (12) found that when the peanut was grown in the light, phosphorus at a concentration ordinarily used in nutrient solutions was toxic but not when the plant was grown in the dark. However, the nitrate ion seems to antagonize, to a degree at least, the toxic effects of the phosphate ion. VOTH and K. C. HAMNER (22) reported that greater growth of *Marchantia polymorpha* occurred in those solutions low in phosphate and high in nitrate. C. L. HAMNER (6) found that if nitrogen was deficient, phosphorus in low concentrations was toxic to the soybean, but that this toxicity disappeared with greater amounts of nitrogen. In general, at least in the younger stages of growth, the toxic effect of two parts of phosphate was overcome by one part of nitrate. BECKENBACH, WADLEIGH, and SHIVE (2) varied the concentrations of both the cations and anions of the nutrient solution. In the case of the anions the solution high in nitrate and low in phosphate and sulphate gave the greatest growth. This is attributed mainly to the effect of the nitrate. They do not discuss the nitrate-phosphate relationship.

As shown by table 2, the nitrogen-phosphorus ratio of solution 5, the plants of which were the largest, is much greater than those of solutions 4 and 3, which ranked second and third. So the relatively low phosphorus content in proportion to the nitrogen may be one reason for the superiority of solution 5. Even this solution is high in phosphorus compared with the concentrations reported optimal by the investigators just mentioned. Solution 5 contains 31 p.p.m. of phosphorus (95 p.p.m. of phosphate). Perhaps the plants would have been larger if the concentration of phosphorus had been even lower.

LOW MAGNESIUM CONTENT

Another factor possibly contributing to the superiority of solution 5 is the high Ca/Mg ratio of this solution in comparison with solutions 4 and 3. As shown by table 2, solution 5 contains less than half as much magnesium as the other solutions. The Ca/Mg ratios of solutions 5, 4, and 3 are, respectively, 4.12, 2.19, and 3.29.

The toxic effects of magnesium and the reduction of the toxicity by calcium are well known. While some have concluded that there must be a rather definite Ca/Mg ratio for satisfactory growth of plants, much of the literature indicates that the ratio may vary widely without injurious effects. LIPMAN (10) gives a comprehensive review of field experiments on the lime/magnesia ratio in soils. He finds little or no support for the idea that it is necessary to have in soils a proper lime/magnesia ratio. Much of the more recent work with solution and sand cultures is to the same effect. BECK-

ENBACH *et al.* (2) found that the Ca/Mg ratio was not significant in the growth of corn, and GAUCH (5) found no well-defined Ca/Mg ratio which was optimal for the growth of bean.

While it may be hard to demonstrate the necessity of a definite Ca/Mg ratio for the growth of plants, this does not mean that the amount of magnesium in the nutrient solution is not important. High concentrations of magnesium result in smaller plants. It is possible that growth of the plants of solutions 4 and 3 was reduced owing to the presence of too much magnesium and that the low magnesium content of solution 5 in proportion to the calcium is one reason for the greater growth of the sunflower in this solution. But this should not be overemphasized. It would seem that solutions 4 and 3 are sufficiently high in calcium to prevent inhibition of growth by the magnesium. However, the antagonism might be incomplete.

HIGH PH OF DRIP FROM THE POTS

As shown by table 3, the pH of the drip of solution 5 ranged 6.71–7.92, higher than any other solution. Three-fourths of the determinations were alkaline. The nitrate content of solution 4 approaches—and of solution 3 exceeds—that of solution 5 (table 2). The first two solutions are much higher in potassium phosphate than solution 5, with the result that the pH of their drip is lower. Before application to the sand, the pH of the solutions did not vary widely.

The greater absorption of cations than anions causes a decrease in pH. Solution 6 contained a small amount of ammonium sulphate, and the pH of its drip does not rise as high and shows less fluctuation than any other solution. TRELEASE and TRELEASE (20) found that with a certain nitrate/ammonium ratio there was very little change in the pH of the nutrient solution.

Even with the high pH of solution 5, growth of the sunflower was not inhibited. The plants were the largest of all the series. They might have been still larger at a lower pH, but their appearance did not indicate any adverse effects of the high pH—such as the deficiency of one or more of the manganese, iron, and phosphate ions. Iron was added as ferric citrate. If other ions, such as manganese or phosphate, were precipitated, it would only be after they were applied to the sand. The original solution was acid enough to keep them in solution. If precipitation occurred in the sand, it would probably be a finely divided one, which on accumulation might dissolve sufficiently to give the plants an adequate supply of the ions.

Summary

1. By means of sand cultures, solution 3 (IR_2S_4 of the LIVINGSTON triangle, osmotic pressure 1.0 atmosphere) was compared in the growth of the sunflower with dilutions of this solution and with solutions of other investigators. Six solutions were compared.

2. The plants grown with solution 5 (HOAGLAND and ARNON) were the largest of any series, 17% larger than those of solution $^{T}R_2S_4$. Its superiority was probably due to its nearly optimal concentration (approximate osmotic pressure 0.7 atm.), its low phosphate content in proportion to nitrate, and its low magnesium concentration in relation to calcium.

3. The plants of solutions 1, 2, and 6 (approximate osmotic pressure 0.25, 0.50, and 0.50 atm., respectively) were smaller than those of IR_2S_4. Expressed in percentages, the range was 44.92% smaller in the case of solution 1 of the

first crop to 8.10% in solution 2 of the same crop, the other differences being between these extremes. These three solutions were probably too dilute for best growth of the sunflower.

4. Solution IR2S4 with an osmotic pressure of 1 atm. seemed too concentrated. The composition of solution 4 was the same as IR2S4, except that the former solution was somewhat lower in calcium nitrate. This would be expected to decrease rather than to increase growth. Yet, probably because of a more optimal concentration, the plants of solution 4 (approximate osmotic pressure 0.9 atm.) were larger by 5.0–9.0% than those of IR2S4.

5. Both solutions 3 (IR2S4) and 4 produced plants inferior to those of 5. Their inferiority was probably due to their higher concentrations and higher contents of phosphate and magnesium.

6. The pH of the drip of solution 5 was higher than that of any other solution, most of the determinations showing an alkaline reaction. This high pH was due to the facts that all the nitrogen was supplied as nitrate and the content of potassium phosphate was quite low. Solution 6 (SHIVE and ROBBINS) contained a little ammonium sulphate, and the pH of its drip did not rise so high and showed less fluctuation than any other solution.

7. Because of higher temperatures and more sunshine, the plants of the second harvest were considerably larger than those of the first. The increased growth of the former plants in comparison with the latter was greater for the roots than for the tops, so that the top-root ratios of the second crop were smaller.

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THERMOREGULATION IN THE EXPERIMENTAL GREENHOUSE

K. STARR CHESTER AND W. WINFIELD RAY

It has been shown by a number of investigators (DODOV, GASSNER and STRAIB, RUDORF, ROBERTS, GESHELE, HASSEBRAUK, NEWTON and JOHNSON, and CHESTER) that the reactions of wheat varieties used in differentiating physiological races of wheat leaf-rust (*Puccinia triticina* Eriks.) may differ so markedly in response to greenhouse temperatures that race identifications by different workers—or by the same worker at different times—are often not comparable. The equipment described here was installed to overcome this and other similar problems in pathological research.

The marked advances in methods for uniformly heating buildings during the past few years offer the possibility of applying the same principles to the experimental greenhouse. In 1943, thermostatically controlled projection steam heaters, such as are used for thermoregulation of institutions and commercial buildings, were installed in two of the experimental greenhouses of the Oklahoma Agricultural Experiment Station. The results were so satisfactory that the essential features of the installations are briefly presented here. The writers will be glad to furnish additional information on details of the installation, operation, and the relatively low cost of the equipment ($200 per house under existing conditions).

The two greenhouses selected were nearly square, with 800 sq. ft. of floor space, height of 15 ft. at the center, and volume of 7000 cu. ft. It was desired to maintain the temperatures of the two houses as constantly as possible at 65° and 80° F., respectively. In each greenhouse, at the center and 13 ft. from the floor, was installed a projection unit heater consisting of a hollow coil of small steam tubes surrounding an electric fan (1150 r.p.m.) which projected the heated air to all parts of the greenhouse by directing it at an anemostat or diffuser having four cone-shaped baffles (fig. 1). At a convenient point midway between heater and outer walls was located a pair of mercoid-type line voltage thermostats, one of which activated the heater fan, the other an alarm bell located at a distant point in the greenhouse system where an attendant was on duty throughout the day. There was no at-

tendant on duty at night, nor was one needed. The steam supply to the standard greenhouse system of heating pipes (which were undisturbed) was reduced to provide the greenhouse with only sufficient heat to warm it to a point approximately 15° F. lower than the temperature desired, and the projection

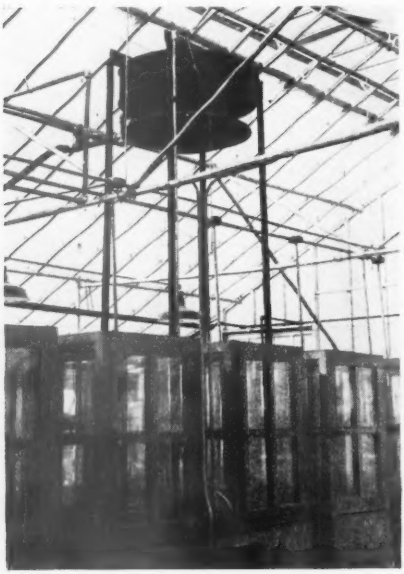

FIG. 1.—Projection steam heater and diffuser installed for thermoregulation of greenhouse. Battery of constant soil-air temperature cabinets shown below.

heater provided the remaining heat required.

The installation was semi-automatic to this extent: the thermostat activated the heater within a 1° limit if the temperature dropped. If, because of the sun's radiation or rising outdoor temperatures, the temperature rose as much as 3° above that desired, the second thermostat activated the alarm bell as a signal for the attendant to open the ventilators. Usually the alarm bell rang once a day, about 2 hours after sunrise, and this was soon forestalled by more prompt opening of the ventilators.

With lack of means for cooling the greenhouses, the equipment is useful only during the period in which outdoor temperatures are 10° or more lower than the desired greenhouse temperatures (approximately October–March for the 65° greenhouse and September–May for the 80° greenhouse under Oklahoma conditions); nevertheless these periods are adequate for an extensive program of greenhouse research during the period in which field experiments are inactive.

The efficiency of the equipment is indicated by the typical thermograph records in figure 2. They show temperatures in the "65°" greenhouse for January 5–12, 1942 and January 10–17, 1944, before and after the thermoregulatory equipment was installed. That outdoor temperatures were similar for the 2 weeks is shown by the dotted lines connecting outdoor maximum and minimum daily temperatures at a point a few rods from the greenhouse during the same periods. With thermostatic regulation, the temperatures for the week did not once depart from the range 62°–68° F., while in the same greenhouse in a comparable week with manual control the temperatures were within this same range only 38% of the time. The slight variations seen in the lower record are principally the result of temperature fluctuations due to the opening of doors and other disturbances in connection with watering and care of the greenhouse.

The efficiency of this means of thermoregulation is also reflected in the results of infection experiments and in the normalcy of plant growth. Prior to installation of the equipment it was not unusual to have 50% of infection tests with wheat leaf-rust result in failure because

of excessively high or low temperatures, particularly in the 6–8 hour period following inoculation. Since the equipment was installed there has not been a single failure from this cause. With such temperature control and 50 candle-power of supplementary light in the 65° greenhouse at night, winter wheat has reached heading stage in slightly more than 2 months after planting, which indicates the possibility of raising two generations of wheat per year in breeding experiments, even during the relatively brief fall-to-spring season in the latitude of Oklahoma.

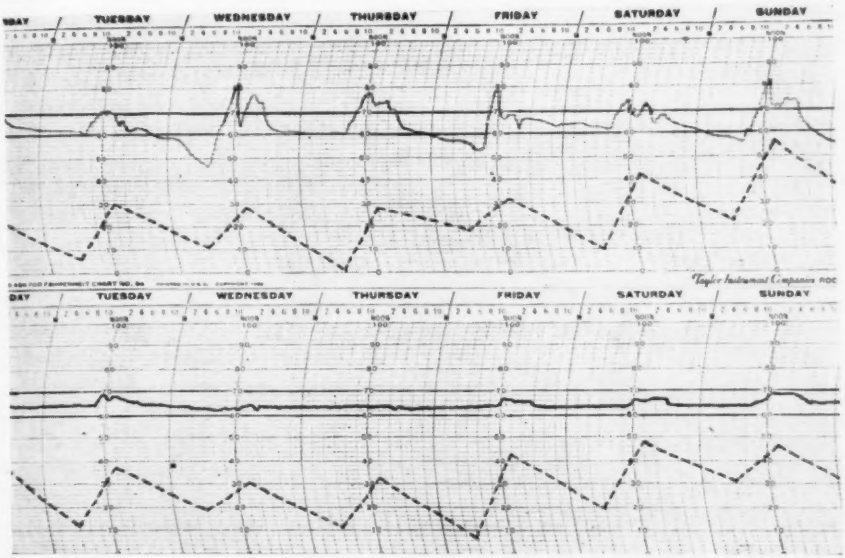

FIG. 2.—Record of attempts to maintain uniform temperature of 65° in greenhouse. Upper, before installation of thermoregulatory equipment (Jan. 5–12, 1942); lower, after installation (Jan. 10–17, 1944). Solid lines show greenhouse temperatures; dotted lines connect daily outdoor maxima and minima.

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A CHAMBER FOR GROWING PLANTS UNDER CONTROLLED CONDITIONS

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A chamber has been devised, illuminated by fluorescent lamps, in which several species of plants have been grown from seed to maturity. In these chambers relatively accurate control is exercised over most of the environmental factors which influence plant growth and development. Other investigators (1, 2) have found the fluorescent type of lamp satis-

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factory as the sole source of illumination for growing plants, in that they are more efficient than tungsten filament types and produce less heat for a given amount

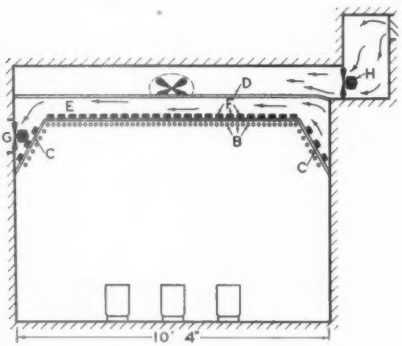

FIG. 1.—Diagrammatic cross-section of control chamber, about 4 feet from end of room near which lighting equipment is placed. The fan (center), which serves to recirculate the air in the room, is shown encircled with broken lines, since it would not fall in the plane of this section. Three pots shown in position.

of illumination. Almost any desired quality may be obtained, but intensities above 2000 foot-candles are impossible to secure.

Description

Figures 1 and 2 show end and side elevations of the chamber and the equipment installed in it. The chamber is a basement room approximately $10\frac{1}{2} \times 16\frac{1}{2}$ feet, with three interior walls of cinder block and an outside (foundation) wall of concrete. Three 2×6 joists were installed across the room, with clearance of $6\frac{1}{2}$ feet from the floor (fig. 2*A*). To these were attached eight light panels of wood, 2×3 feet, each carrying twelve 30-watt, 36-inch fluorescent tubes on the lower side and the accessory equipment for the tubes above. The lower surfaces of the panels were painted white, as reflectors, and the upper surfaces were covered with heavy asbestos as a protection between the wood and the accessory equipment. This installation formed a false ceiling 6×8 feet near one end of the room and equidistant from the side walls. Four similar panels, each carrying six fluorescent tubes, were installed between the edge of this false ceiling and the side walls of the room at an angle of about 60° (fig. 2*C*) with the wall; and finally, two 6-tube panels were suspended at

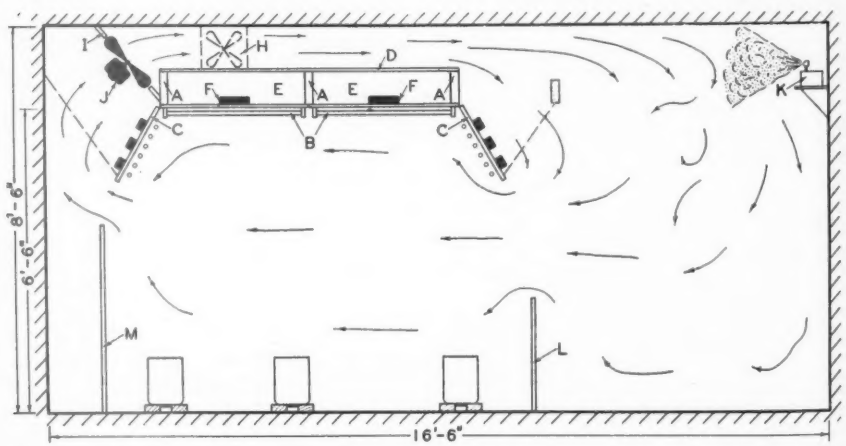

FIG. 2.—Diagrammatic longisection of control chamber. Fan *H*, which controls entrance of outside air, shown in broken lines to indicate its relative position. Three pots shown in position.

each of the other two edges of the false ceiling, at an angle of about 45° with the vertical. Thus, the light source consisted of 144 30-watt fluorescent tubes.

Plywood (fig. 2*D*) across the top of the joists holding the lamp panels formed two horizontal ducts (*E*) between the joists, the floor of the ducts consisting of the lamp panels, and with the accessory equipment for the tubes attached to this floor. The ducts were extended to the side walls of the room by the use of plyboard, and openings were provided at the ends of the ducts so that air from the chamber could be drawn through them and exhausted from the room through a hole in the side wall by a fan (fig. 1*G*), thus removing most of the heat generated by the accessory equipment of 122 of the lamps. The exhaust fan ran all the time the lights were on.

Just above the ducts, and at the wall opposite the exhaust fan, an intake fan (fig. 1*H*) was installed at an opening to the outside of the building. It was thermostatically controlled to draw in cool outside air whenever the room temperature rose to a given point.

A plywood sheet (fig. 2*I*) was placed at an angle of about 45° from the horizontal lamp panels to the ceiling of the chamber, blocking communication between air above and below the lamp and duct assembly at the end of the chamber.

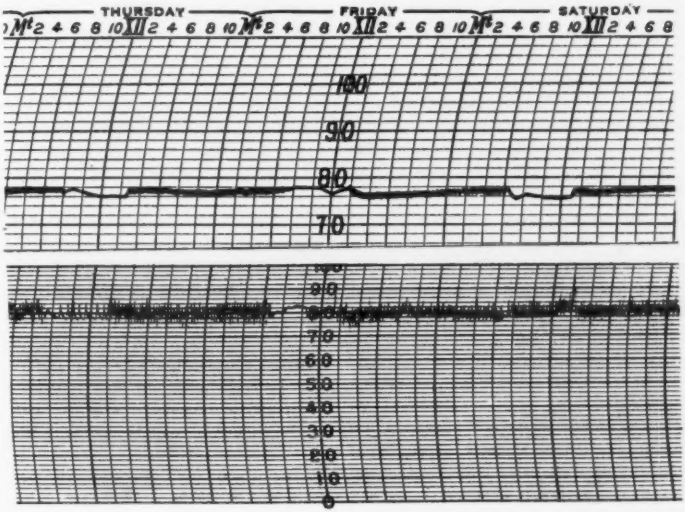

FIG. 3.—Portion of typical weekly hygrothermograph page. Above, temperature graph; below, graph of percentage relative humidity.

A 14-inch fan (*J*) was installed in this barrier and operated continually to circulate the air in the chamber.

A white-painted baffle (fig. 2*L*) 2 feet high was set on the floor, across the chamber opposite the circulating fan *J* and just beyond the location of the lamp panels. A second baffle (*M*) was installed similarly beneath the fan. These served as reflectors and produced eddy currents that insured thorough air circulation throughout the chamber. Near the ceiling at the end of the room opposite the lamp arrangement was installed a Spray-

co[2] humidifier (*K*) with three nozzles activated by compressed air. When actuated by a horse-hair hygrostat located near the recirculating fan *J*, this humidifier delivered moisture as a fine fog that evaporated almost at once.

Discussion

In preliminary work, many types of fluorescent tubes were tried to determine what quality of light was most satisfactory, and it was found that the best growth of the plants tried was obtained through the use of white fluorescent tubes alternating with the daylight tubes.

FIG. 4.—Tomato plant 11 weeks old, grown from seed in control chamber continuously. Some of the fruit from this plant had been harvested for analytical purposes.

Three chambers of this kind have been in actual operation for more than a year, with satisfactory results. Figure 3 shows a portion of a weekly hygrothermograph record from one of the rooms. In this particular case the lights burned for approximately 15 hours each day, and in the remaining 9 hours the rooms were in darkness. It has been our experience that more nearly uniform records are obtained when the chambers are illuminated continuously. During the coldest months of the year it has been necessary to supply supplementary heat during the hours of darkness if the temperature of the chamber is to be maintained fairly high. This may be accomplished through the use of small electric heaters or any other heating system which will operate continuously during the dark period.

Plants of Biloxi soybean, red kidney bean, and tomatoes have been grown successfully from seed to maturity, and many other plants have been grown satisfactorily during experiments which did not involve seed production. Figure 4 shows a tomato plant which was grown in the room for 11 weeks at a temperature of $77° \pm 1°$ F. and a relative humidity of $80 \pm 5\%$. Many of the fruits had been removed from this plant for analytical purposes. A fairly heavy crop was produced and ripened satisfactorily. Practically all the leaves were still present, and nearly all were of a uniform deep-green color. In appearance these plants were comparable with those of the same age which had been grown in the greenhouse during the late spring. Plants grown in these chambers from November to April have always produced nearly twice as much vegetation as those in the greenhouse at Ithaca, New York. Outdoors during the summer months, tomato plants produced about 30% more dry weight and about 50% more fruit than did those in the control room. The temperature during the dark period was fairly high in the control chamber, however, and it might have been possible to grow

[2] Spray Engineering Co., Somerville, Massachusetts.

plants more nearly comparable with those outdoors if other temperatures had prevailed. This would have been true more particularly if the temperature during the dark periods had been lowered.

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ANATOMICAL RESPONSES OF TOMATO STEMS TO VARIATIONS IN THE MACRONUTRIENT CATION SUPPLY

C. B. LYON[1] AND C. R. GARCIA[2]

Introduction

In a previous study (6), variations in nitrate, sulphate, and phosphate supplied to tomato plants grown in sand culture resulted in differences in the anatomy of tomato stems. Cellular differences which could be associated with nutrient supply were described. Differences in the actual—as well as the relative—area of component tissue systems in stem cross-sections were reported, and these characteristics were correlated with growth and fruitfulness of the plants.

It is the purpose of this paper to complete the survey of anatomical differences in tomato stems which result from variations in the relative proportions of macronutrient ions supplied and to correlate observed differences with the vegetative and fruitful condition of the plant. The results with respect to variations in the calcium, potassium, and magnesium supply are reported.

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Material and methods

The material was obtained from the 1941 experiment of HAMNER, LYON, and HAMNER, described in detail elsewhere (4). Briefly, an inbred strain of Bonny Best variety of tomatoes was planted in the greenhouse May 12, 1941, and the seedlings were transplanted to 2-gallon glazed crocks containing pure quartz sand. On July 1, all plants were placed outdoors. The plants were harvested September 10.

The design of the experiment was that of a randomized block (2) with eighty-seven treatments and four replications. Each replication consisted of a three-plant row, and the replications were randomized by the use of TIPPETT'S randomization tables (8). Thus, the mean of twelve plants is used as an estimation of the results produced by a given treatment. The data were reduced by means of the analysis of variance, and the t test (7) was used for determining whether particular differences were significant. Odds as great as or greater than 99:1 were accepted as statistically significant that the observed

deviations were not due to errors of random sampling. Subsequent to the time the seedlings were transplanted, the same randomization and design was maintained, both in the greenhouse and in the field.

In the forty-three solutions considered in this investigation, the relative proportions of calcium, potassium, and magnesium were varied, while the total equivalent concentration of these ions was constant. Each solution contained 12.0 milliequivalents per liter of nitrate, 4.5 me./l. of phosphate, and 9.0 me./l. of sulphate. For convenience, the forty-three solutions are represented in a cation triangle (fig. 1), and the treatment numbers are assigned to those solutions used. All solutions contained equal amounts of the micronutrients in the following concentrations:

B (as H_3BO_3)	0.5	p.p.m.
Mn (as $MnCl_2 \cdot 4H_2O$)	0.5	
Zn (as $ZnSO_4 \cdot 7H_2O$)	0.05	
Cu (as $CuSO_4 \cdot 5H_2O$)	0.02	
Fe (as ferric citrate)	5.0	

When the plants were 120 days old and had received their respective nutrient treatments for 94 days they were harvested, and a section of the stem from the middle internode of each plant was preserved for anatomical study. The stem sections were fixed with Navashin's solution, and air was evacuated from the tissues. The material was dehydrated in an ethyl-tertiary butyl-alcohol series and infiltrated with a paraffin-beeswax rubber mixture. Complete cross-sections were cut at 10–20 μ and stained with a modified Flemming's triple stain. The material was mounted in balsam.

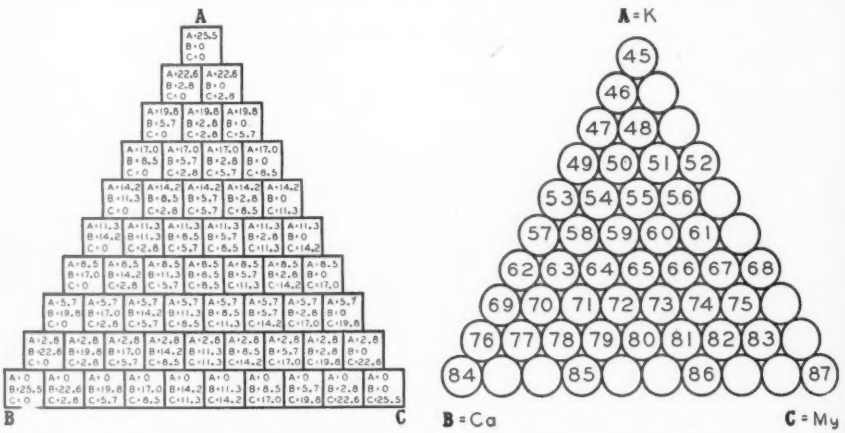

FIG. 1.—Left, macronutrient cation composition (me. /l.) of nutrient solution for 55 possible treatments. Right, treatments used, with treatment numbers assigned.

All stem sections were projected at a constant magnification of approximately 30 diameters. The images were centered on a screen marked off with four diameters dividing the stem section into eight equal sectors, as previously described (6). The radial measurements of each tissue system on each of the eight radii were recorded. Thus, the mean of eight measurements was used for xylem, phloem, and cortex; the mean of four measurements was used for pith and stem diameter.

In these measurements the internal

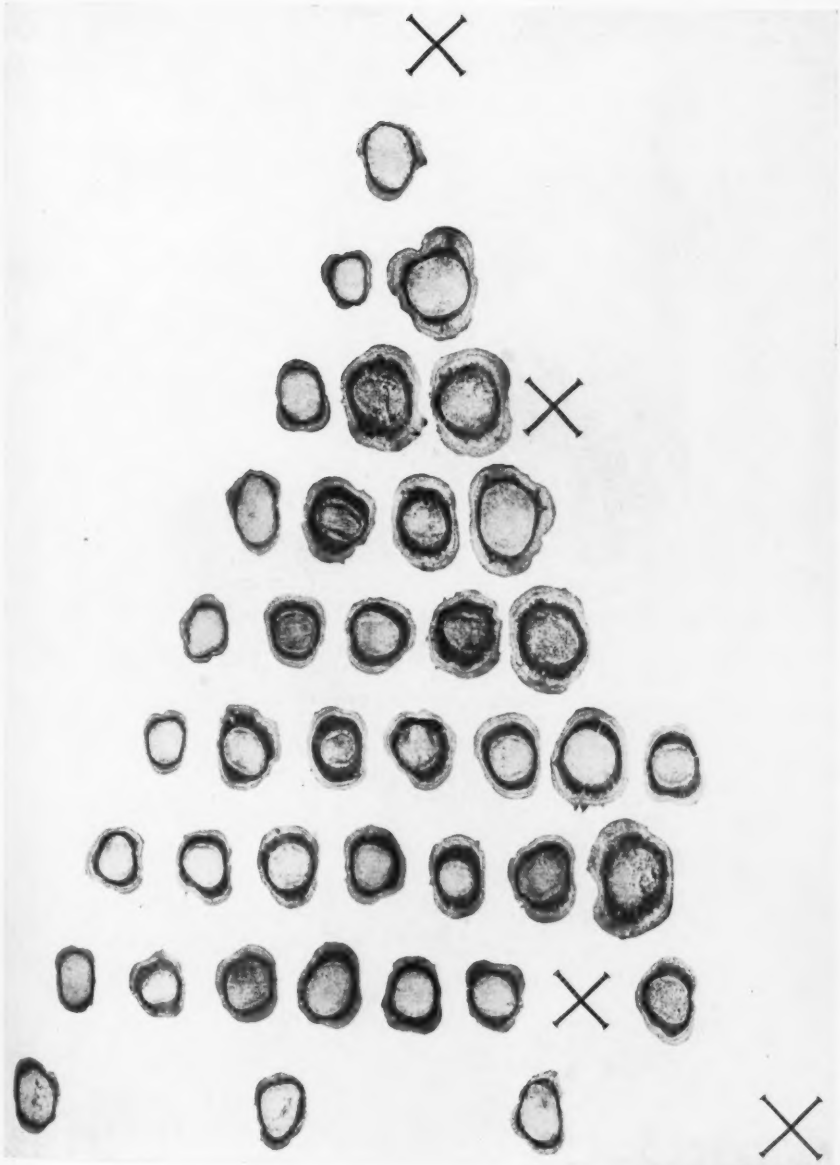

FIG. 2.—Typical cross-sections of tomato stems from plants in cation treatments (corresponding to those shown in fig. 1).

phloem was disregarded, and the term "pith" includes all cells internal to primary xylem; "xylem" includes all cells from primary xylem to cambium; "phloem" includes all cells from cambium to pericycle; "cortex" includes all cells from endodermis to and including cuticle.

The mean radial measurement of any tissue system for a given stem section was used to calculate the actual area of that system in the cross-section. Standard formulas developed for circles were employed. It was considered that the variability within a stem attributable to the deviations of the tissue systems from circles was nullified by the use of mean values. Thus, all inter-treatment comparisons consider only differences between stems with respect to areas of the tissue systems. Two types of such comparisons were made for each tissue system: (*a*) actual areas were computed and treatment means were compared, and (*b*) the percentage cross-sectional area of the stem section occupied by the area of any given tissue system was computed and treatment means compared. In this way the actual size as well as the relative amount of tissue in a stem section was evaluated.

Results

Photomicrographs of cross-sections from typical tomato stems in each treatment are given in figure 2. Large differences—which include stem size, tissue system development, starch accumulation, and to some extent cellular anatomy—are demonstrable. These characteristics will be discussed individually.

This experiment had been specifically designed so that data obtained for any character could be analyzed for the presence or absence of statistically significant differences. Accordingly, analyses of variance were computed for each individual character, and the results are compiled in table 1. Plants from several treatments supplied with nutrient solution lacking calcium were discarded owing to the complete deterioration of terminal meristems, and several plants were discarded as a result of mechanical injury. This resulted in data which were non-orthogonal. The sources of variation are:

TABLE 1

F VALUES FROM ANALYSES OF VARIANCE FOR ANATOMICAL CHARACTERS OF TOMATO STEM CROSS-SECTIONS

Anatomical characters	Source of variation		
	Between treatments	Between blocks	Within blocks
Percentage			
Cortex	12.67*	0.86	0.77
Phloem	25.74*	0.31	1.51
Xylem	18.40*	0.80	4.98*
Pith	22.64*	0.64	5.84*
Area			
Cortex	20.97*	2.23	0.01
Phloem	34.24*	2.48	0.52
Xylem	24.10*	2.52	0.93
Pith	6.19*	1.55	1.53
Stem diameter	20.07*	2.42	0.24

* Highly significant, satisfying statistical requirements for P = 0.01.

Variation due to	No. of degrees of freedom
Treatments	38
Between blocks	3
Within blocks	2
Error	374
Total	417

The substitution of missing values by YATES' method (9) did not significantly change the results of analyses (table 1), and they are considered valid.

It is evident that treatments produced statistically significant differences in all the characters listed. It is also evident

that differences in environment, when measured by replications within blocks, produced significant differences in percentage cross-sectional area occupied by xylem and pith. Since replication differences were measurable in the analysis of variance, and since these differences were not included in inter-treatment comparisons, valid differences exist in all the listed characters which can be ascribed directly to the treatments.

FIG. 3.—Inter-treatment comparison of stem diameters. Treatment no. 48 with stem diameter of 12.0 mm. is designated 100 and all others related to it. Treatment means, together with standard errors, given.

An inter-treatment comparison of stem diameters is given in figure 3. In general, plants from treatments supplied with small relative amounts of calcium in the nutrient medium (2.8 me./l.) had the largest stems of any in the experiment, and stem diameter was significantly less with increments of calcium supply. In the central portion of the triangle there is also a suggestion that stem diameter increases with increments of potassium supply, but no correlation with magnesium supply is evident. Stem size was significantly less than optimum in all calcium-, potassium-, and magnesium-deficient treatments. Supporting evidence for these viewpoints is furnished by a statistical analysis of the data in a manner proposed by BEESON *et al.* (1). Disregarding those treatments in which the nutrient medium was lacking in one or more ions, several significant correlations are evident between nutrient ion supply and stem diameter. If the supply of $K^+ = a$, $Ca^{++} = b$, $Mg^{++} = c$, and stem diameter = v; then:

$$r_{av} = +0.70^{3}$$
$$r_{bv} = -0.80^{3}$$
$$r_{cv} = +0.12.$$

In the same treatments, therefore, potassium ion supply is significantly and positively correlated with stem diameter, and calcium ion supply is significantly and negatively correlated with stem diameter. Magnesium ion supply is not correlated with stem diameter in any significant manner. Since symmetry is a condition of the experiment, the correlations are valid to illustrate trends in the data. It should be emphasized, however, that these correlations do not include data from completely deficient treatments. If such data are included, the magnitude of the correlation coefficients is slightly changed but their significance is not.

An inter-treatment comparison of the actual areas of each of the component tissue systems is given in figure 4. The data for cortex, phloem, xylem, and pith in the central portion of the triangles are very similar, showing maximum development of each tissue system in treatments supplied with small amounts of calcium (2.8 me./l.) in the nutrient medium. As greater amounts

[3] Highly significant, satisfying statistical requirements for P = 0.01.

of calcium are supplied to the plants, the area of each of the component tissue systems is decreased. Table 2 shows the correlations involving calcium ion supply and areas of each of the component tissue systems. The correlations are all negative, significant, and of the same order of magnitude. In addition, both sets of data (fig. 3; table 2) show significant increases in the area of each of the component tissue systems to be associated with increments of potassium ion supply. The correlation coefficients are all positive and significant. No significant relationship involving magnesium ion supply is evident. The complete lack of either magnesium or potassium in the nutrient medium has resulted in development of cortex, phloem, and xylem tissues which have considerably less area than the area of those in optimum treatments. The area of pith is also depressed by a lack of magnesium or potassium but to a somewhat

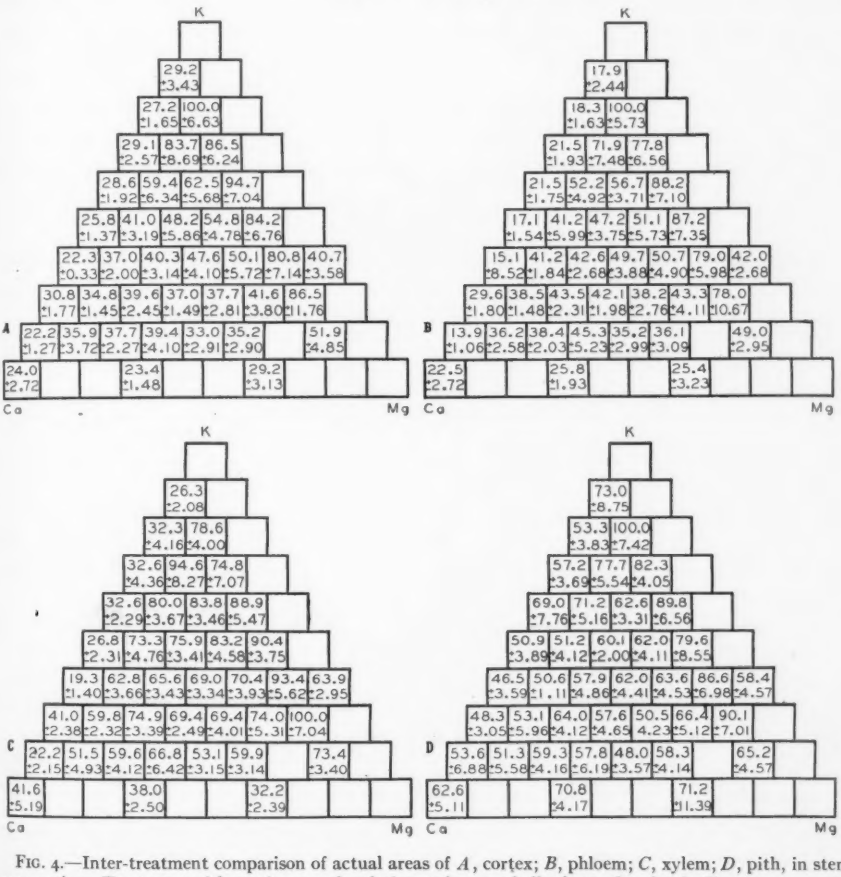

FIG. 4.—Inter-treatment comparison of actual areas of *A*, cortex; *B*, phloem; *C*, xylem; *D*, pith, in stem cross-sections. Treatment with maximum value designated 100 and all others related to it. Treatment means, together with standard errors, given.

less extent than is the case for the other tissues. Lack of calcium supply has resulted in less pith and xylem and considerably less phloem and cortex development than was observed in optimum treatments.

TABLE 2

CORRELATION COEFFICIENTS BETWEEN CHARACTERISTICS OF STEM ANATOMY AND VARIABLES OF NUTRIENT SUPPLY

VARIABLES OF NUTRIENT SUPPLY	AREA			
	Cortex	Phloem	Xylem	Pith
K^+	+0.731*	+0.697*	+0.573*	+0.604*
Ca^{++}	−0.772*	−0.744*	−0.762*	−0.741*
Mg^{++}	+0.056	+0.062	+0.213	+0.157

* Highly significant, satisfying requirements for P = 0.01

As would be expected, the trends with respect to stem diameters are similar to those for areas of component tissue systems. From table 3 it is evident that the areas of various tissue systems are highly significant and positively correlated among themselves and that similar correlations exist between each of the component tissue systems and stem diameter. The magnitude of the correlations is unusually high. Thus, an increase in cortical area as a result of treatment is accompanied by a corresponding increase in the area of phloem, xylem, and pith, as well as a corresponding increase in stem diameter and hence in cross-sectional area of stems. It is noticeable that the correlation coefficient in which areas of xylem and pith are involved as variables is significantly less in magnitude according to FISHER'S Z test (3) than all other coefficients.

Differences in the percentage cross-sectional area of the stem section occupied by the area of each of the constituent tissue systems are shown in figure 5. Relatively small but statistically significant differences are evident for each characteristic when treatments represented in the central portion of the triangle are compared. For instance, area of cortex varied from 17.5 to 26.9%; phloem, 12.4 to 18.0%; xylem, 22.0 to 36.5%; cortex, 29.1 to 37.3%. Since these differences are small, it is difficult to visualize trends associated with the supply of individual ions in the nutrient medium. The trends, however, can be illustrated in the data of table 4. Two contrasting situations are evident. Increases in the percentage area of either cortex or phloem are significantly and positively correlated with increments of potassium ion supply and significantly and negatively correlated with increments of calcium ion supply. No significant trends are associated with magnesium ion supply. On the other hand, increases in the percentage area of either xylem or pith are negatively and significantly correlated with increments of potassium ion supply and positively and significantly with increments of calcium ion supply. As before, no significant trends are associated with magnesium ion supply. Thus, not only stem diame-

TABLE 3

CORRELATION COEFFICIENTS BETWEEN VARIABLES IN CROSS-SECTIONS OF TOMATO STEMS

ANATOMICAL CHARACTERS	AREA			STEM DIAMETER
	Phloem	Xylem	Pith	
Area*				
Cortex	+0.974†	+0.813†	+0.820†	+0.962†
Phloem	.....	+0.871†	+0.769†	+0.978†
Xylem	.....	.....	+0.523†	+0.915†
Pith	.....	.....	.....	+0.800†

* Actual area of indicated tissue system in stem cross-section.

† Highly significant, satisfying requirements for P = 0.01.

ter and the actual area of each of the component tissue systems, but the relative area of each tissue system in the cross-section as well, are significantly correlated with both potassium and ment of cortex, phloem, and xylem but accentuates the relative development of pith tissue. This fact should support a negative relationship between the relative area of cortex, phloem, or xylem

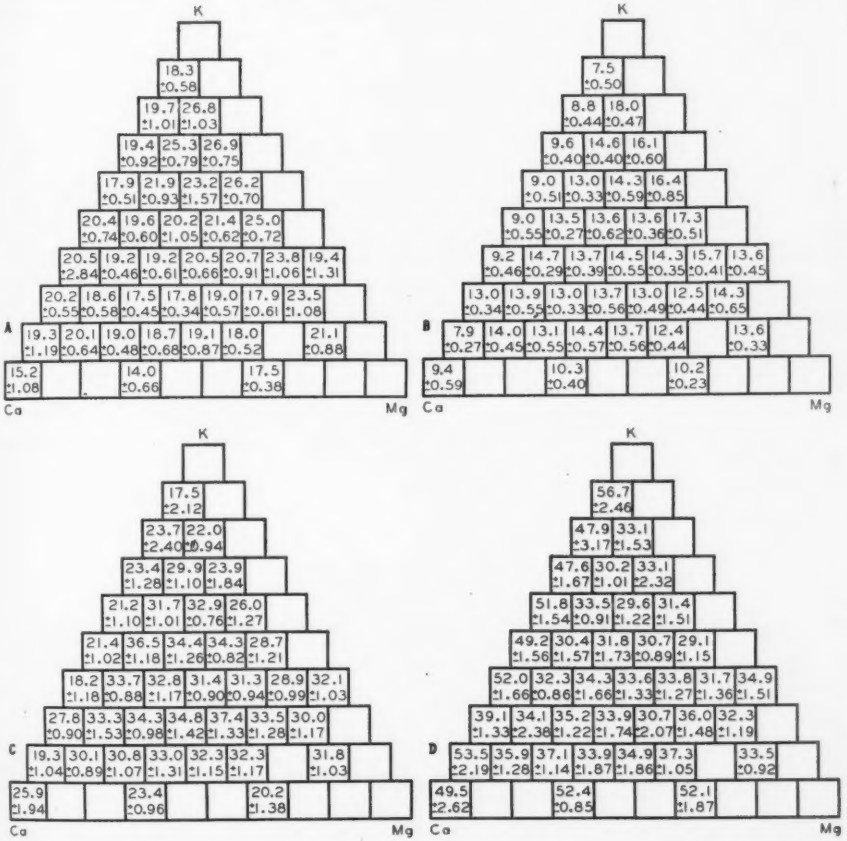

FIG. 5.—Percentage cross-sectional area of stem section occupied by area of *A*, cortex; *B*, phloem; *C*, xylem; *D*, pith. Treatment means, together with standard errors, given.

calcium ion supplies. However, the sign and magnitude of the correlation coefficients differ, depending on the characteristic under consideration.

A complete lack of potassium or magnesium ions in the nutrient medium subsequent to the seedling stage depresses to varying extents the relative develop- and pith. That such is actually the case is shown in table 5. Highly significant and negative correlation coefficients between percentage cortex, phloem, or xylem and percentage pith are evident. Statistically the magnitude of the correlation coefficient involving percentage cortex is significantly less than either of

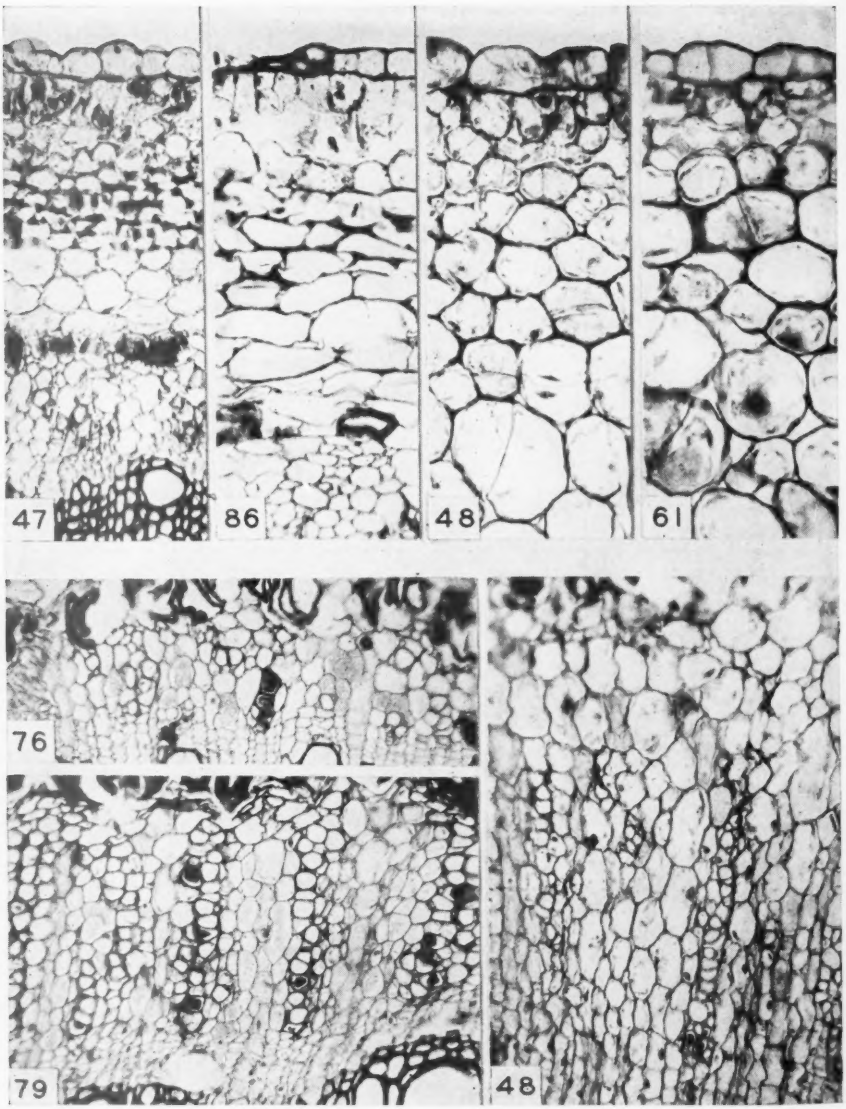

FIG. 6.—Top, series of transections of cortical sectors (external to medium-sized vascular bundles) in stems from indicated treatments; × 128. Bottom, series of transections of external phloem of comparable vascular bundles in stems from indicated treatments; × 141.

those involving percentage phloem or xylem. This may in part be due to the relatively small variation between treatments with respect to percentage cortex. Although the actual area of each of the component tissue systems increases with increased stem diameter, the relatively greater development of vascular tissue (and to a less extent of cortical tissue) is made at the expense of pith. Percentage phloem is significantly and positively correlated with both percentage cortex and percentage xylem, although the magnitude of the coefficients is not large. No significant correlation between percentage cortex and percentage xylem is evident.

TABLE 4

CORRELATION COEFFICIENTS BETWEEN CHARACTERISTICS OF STEM ANATOMY AND VARIABLES OF NUTRIENT SUPPLY

Variables of nutrient supply	Percentage area			
	Cortex	Phloem	Xylem	Pith
K^{+}	+0.778*	+0.607*	−0.520*	−0.602*
Ca^{++}	−0.710*	−0.479*	+0.467*	+0.506*
Mg^{++}	−0.059	−0.127	+0.049	+0.090

* Highly significant, satisfying requirements for P = 0.01.

Extreme variation in the organization, size, and degree of differentiation of constituent cells in each tissue system was evident. Figures 6 and 7 show extremes observed in the development of cortex, external and internal phloem, and pericycle. For convenience, each photomicrograph is labeled with its treatment number, which will be used for reference in the following discussion.

The development of cortical chlorenchyma was clearly related to the supply of magnesium ion in the nutrient medium. In all magnesium-deficient treatments, two to five layers of small cells densely packed with chloroplasts were present immediately beneath the epidermis (fig. 6, no. 47). The development of chlorenchyma in magnesium-deficient treatments was not altered by variations in the supply of potassium and calcium to the plant. In treatment 48, supplied with 2.8 me./l. of magnesium, all cortical cells were larger than in magnesium-deficient treatments, but the development of chlorenchyma was relatively less extensive, in that the number of cell layers was less. In treatment 61, supplied with 14.2 me./l. of magnesium, chlorenchyma consisted of one to two layers of cells, and cortical tissue was predominantly large, succulent parenchyma. Thus, as magnesium supply to the plant was increased, the number of cell layers comprising chlorenchyma was less, and the number—and to some extent size—of parenchymatous cells adjacent to the endodermis increased. In all potassium-deficient treatments the chlorenchyma was restricted to one to two cell layers, and the cells were columnar in shape, with the number of plastids minimized (no. 86).

TABLE 5

CORRELATION COEFFICIENTS BETWEEN VARIABLES IN CROSS-SECTIONS OF TOMATO STEMS

Anatomical characters	Percentage area		
	Phloem	Xylem	Pith
Percentage*			
Cortex	+0.649†	−0.050	−0.523†
Phloem	.....	+0.551†	−0.904†
Xylem	.....	.....	−0.812†

* Percentage cross-sectional area of stem section occupied by area of indicated tissue system.

† Highly significant, satisfying requirements for P = 0.01.

On the other hand, collenchyma was related primarily to calcium supply. In

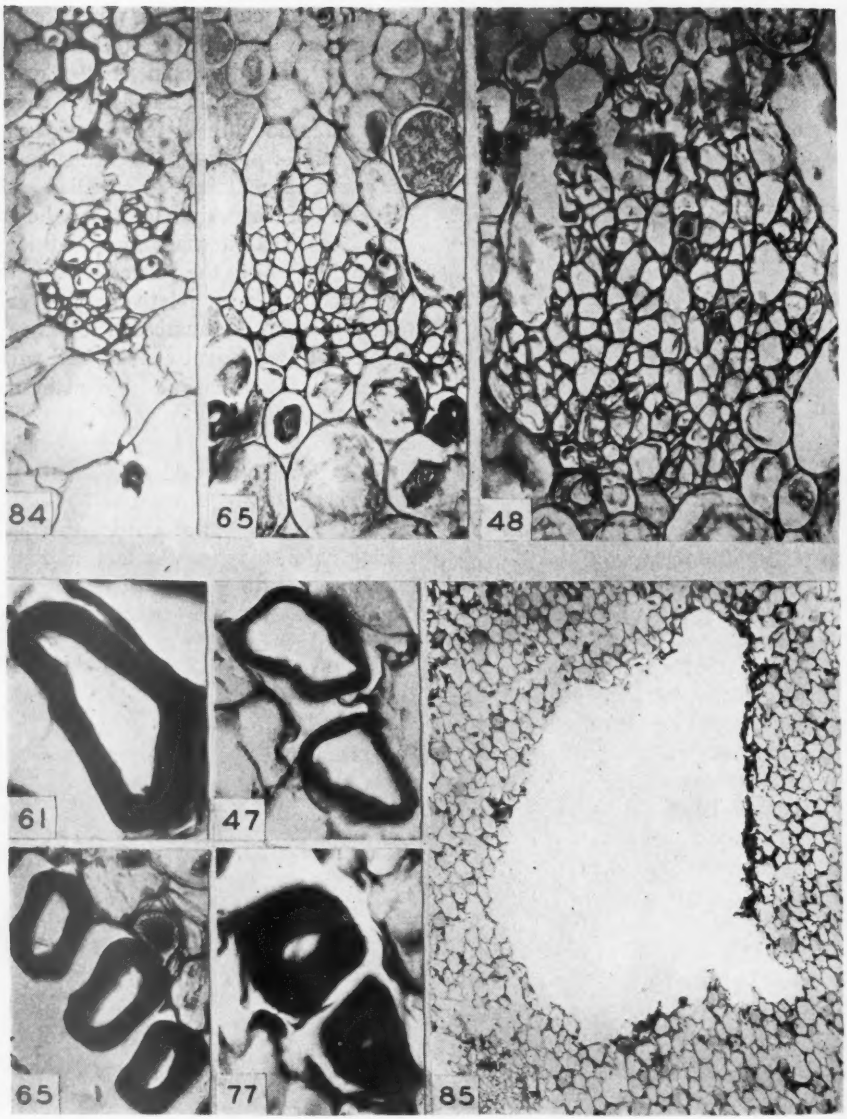

FIG. 7.—Top, series of transections showing internal phloem centrad to primary xylem points of comparable vascular bundles in stems from indicated treatments; × 192. Bottom left, representative pericyclic fibers in stem cross-sections from indicated treatments; × 432. Bottom right, transection through pith in stem from indicated treatment; × 20.

treatments supplied with 2.8 me./l. of calcium, collenchyma consisted of one to two layers of large cells with little or no thickening of the cell walls (nos. 48 and 61). As calcium supply was increased, smaller cells were evident, but the number of cell layers comprising collenchyma as well as the relative wall thickness increased. In all potassium-deficient and magnesium-deficient treatments collenchymatous cells were the smallest of any in the experiment, but cell walls were comparatively thickened (no. 47).

The characteristics of external phloem tissue appeared to be most strikingly associated with potassium supply, although some influence of calcium supply was apparent. In treatment 48 (fig. 6), which was supplied with large amounts of potassium, secondary phloem consisted primarily of large succulent parenchymatous cells with relatively infrequent sieve tubes and companion cells. As potassium supply was successively less, a greater differentiation of parenchyma into sieve tubes and companion cells was evident. The maximum relative development of sieve tubes and companion cells occurred in treatments supplied with low amounts (2.8 me./l.) of potassium (no. 79) and relatively high amounts of calcium. In other words, low potassium supply was associated with extensive differentiation of secondary phloem parenchyma into sieve tubes and companion cells, but within these treatments high calcium and low magnesium supplies were associated with maximum differentiation, and this condition was evident at each level of potassium supply. In treatments at the high-calcium apex of the triangle, it was evident that some parenchyma cells of secondary phloem in stem sections were packed with crystals—supposedly calcium oxalate (nos. 76 and 79). The number of crystal-containing cells and the amount of crystals in each cell increased with increments of calcium supply. In all magnesium-deficient treatments secondary phloem consisted primarily of parenchyma cells with isolated groups of three to five sieve tubes and companion cells. Some crushing of primary phloem was evident (no. 76). There was large variation in secondary phloem development in potassium-deficient treatments. The range was from large succulent parenchyma with small isolated groups of sieve tubes and companion cells in treatments supplied with relatively low amounts of calcium and high amounts of magnesium to highly organized sieve tubes and companion cells with sparse parenchymatous cells in treatments supplied with relatively high calcium and low magnesium.

The most extensive internal phloem development—using number of internal phloem bundles and size and number of sieve tubes and companion cells in each bundle as criteria—occurred in treatments supplied with 2.8 me./l. of magnesium (fig. 7, no. 48). Less extensive internal phloem development was associated with increased magnesium supply (no. 65). At any given level of such supply, however, the most extensive internal phloem development occurred in treatments supplied with relatively large amounts of potassium and low amounts of calcium. A complete lack of any macronutrient cation in the nutrient medium —magnesium, potassium, or calcium— resulted in minimum amounts of internal phloem (no. 84).

The largest internal and external pericyclic fibers with relatively thin walls occurred in treatments supplied with 2.8 me./l. of calcium (no. 61). As calcium supply was increased, the size

of fibers decreased and the relative wall thickness increased (no. 65). Maximum wall thickness and minimum cell size were associated with high calcium supply (no. 77). At any given level of calcium, no effect of magnesium or potassium supply was evident. Medium to large-sized fibers with minimum wall thickness were observed in treatments lacking one or more ions in the nutrient.

TABLE 6

RELATIVE COMPARISON OF EFFECTS OF THREE CONDITIONS OF NUTRIENT SUPPLY ON CELLULAR CHARACTERISTICS IN CROSS-SECTIONS OF TOMATO STEMS

CHARACTER	TREATMENT NUMBER		
	77 (high calcium)	48 (high potassium)	83 (high magnesium)
Cortex			
Chlorenchyma			
No. cell layers	++*	+++	+
Plastid development	++	+++	+
Collenchyma			
No. cell layers	+++	+	++
Cell size	+	++	+++
Wall thickness	+++	+	++
Cortical parenchyma			
No. cell layers	+	++	+++
Cell size	+	+++	++
Pericyclic fibers			
Cell size	+	+++	+++
Wall thickness	+++	+	+
External phloem			
Cell size	+	+++	+++
Relative amount of parenchyma	+	+++	++
Relative amount of sieve tubes and companion cells	+++	+	++
Crystal inclusions in parenchyma	+++	.....	.....
Internal phloem			
Cell size	++	+++	+
No. strands	++	+++	+
No. cells per strand	++	+++	+

* Development: + = minimum; ++ = intermediate; +++ = maximum.

Several distinguishing characteristics of pith cells were evident. In all potassium-deficient treatments, complete disintegration of central pith cells had occurred, while the remaining cells were small and thin-walled. The small size of pith cells in all magnesium-deficient and calcium-deficient treatments was evident, but no disintegration of central cells occurred. Some variation in size and wall thickness of pith cells in treatments in the central portion of the triangle was evident, but the differences could not be clearly correlated with variations in nutrient supply. However, some parenchymatous cells from treatments supplied with relatively large amounts of calcium were filled with granular inclusions. The occurrence of these inclusions in pith cells was closely correlated with their occurrence in phloem parenchyma.

Certain trends with respect to cellular anatomy are thus clearly correlated with nutrient supply. These trends are crudely represented in table 6. In this table three conditions of nutrient supply were arbitrarily established, and the responses associated with the complete lack of any ion in the nutrient medium were disregarded, since they would have little practical significance. It should be recognized that plants in treatment 77, denoted "high calcium," were supplied with relatively low amounts of potassium and magnesium ions in the nutrient. The responses noted are therefore associated with the relative supply of all three ions, rather than with the supply of any single ion.

In this experiment, significant and large differences occurred between treatments with respect to stem diameter and the actual area of each of the component tissue systems in the stem sections. Small but significant differences between treatments in the relative pro-

portions of the tissue systems in the stem were also evident. In a previous experiment (6), the actual area as well as the percentage area of certain tissue systems (notably vascular tissue) was correlated with the vegetative growth and fruitful conditions of the plant. In that experiment, maximum vegetative growth and maximum fruitfulness occurred in identical treatments. Since in the present experiment maximum vegetative growth occurred in treatments supplied with relatively small amounts of calcium, while maximum fruitfulness was obtained in treatments supplied with relatively large amounts of calcium (4), it is of interest to examine similar correlations. The results of the correlation analysis are given in table 7.

TABLE 7

CORRELATION COEFFICIENTS BETWEEN ANATOMICAL CHARACTERISTICS OF TOMATO STEMS AND CHARACTERS USED AS CRITERIA OF GROWTH AND FRUITFULNESS

Anatomical characters	Growth				Fruitfulness		
	Ht. of vine	Fresh wt. of vine	Dry wt. of vine	Dry wt. of roots	Total no. fruit/vine	Fresh wt. fruit/vine	Ave. fresh wt. mature fruit
Percentage*							
Cortex	+0.613‡	+0.566‡	+0.625‡	+0.813‡	−0.260	−0.352	−0.350
Phloem	+0.686‡	+0.824‡	+0.863‡	+0.850‡	+0.320	+0.259	+0.139
Xylem	+0.518‡	+0.564‡	+0.645‡	+0.323	+0.879‡	+0.784‡	+0.639‡
Pith	−0.771‡	−0.828‡	−0.916‡	−0.765‡	−0.584‡	−0.470‡	−0.338
Area†							
Cortex	+0.645‡	+0.683‡	+0.763‡	+0.891‡	−0.185	−0.293	−0.383
Phloem	+0.662‡	+0.761‡	+0.825‡	+0.905‡	−0.039	−0.133	−0.247
Xylem	+0.771‡	+0.810‡	+0.930‡	+0.815‡	+0.347	+0.195	+0.044
Pith	+0.259	+0.362	+0.400	+0.596‡	−0.502‡	−0.565‡	−0.674‡
Stem diameter	+0.665‡	+0.745‡	+0.833‡	+0.875‡	−0.008	−0.130	−0.274

* Percentage cross-sectional area of stem section occupied by area of indicated tissue system.
† Actual area of indicated tissue system in stem cross-section.
‡ Highly significant, satisfying requirements for P = 0.01.

These data show stem diameter and the actual area of each of the component tissue systems, except pith, to be positively and significantly correlated with characteristics used as criteria of vegetative growth. This means that each unit increase in stem diameter or in the cross-sectional area of cortex, phloem, or xylem was accompanied by a unit increase in the vegetative growth of the plants. However, similar anatomical changes could not be correlated in any significant manner with fruitful conditions. The area of the pith was not significantly correlated with growth of the vine but was positively correlated with growth of the roots and negatively with fruit production. The magnitude of those correlations involving area of xylem and growth of the vines as variables is relatively high.

Changes in the relative proportions of each of the constituent tissue systems were significantly correlated with the vegetative and fruitful condition of the plant. Increased relative proportions of cortex, xylem, and phloem in stem sections were significantly correlated with

increased vegetative growth. The magnitudes of all correlation coefficients involving percentage phloem as a variable are significantly higher as measured by FISHER'S Z test (3) than corresponding coefficients involving percentage cortex or percentage xylem. Since the relatively greater increases in the percentage cortical and vascular tissue in the cross-sections were apparently made at the expense of pith (table 5), the correlations involving percentage pith and vegetative growth characteristics as variables are all negative with respect to sign. However, a different situation existed with respect to fruitfulness. The relative extent of cortex and phloem in the stem sections was not significantly correlated with characteristics used as criteria of fruitfulness, although highly significant and positive correlations existed when percentage xylem was considered. Thus, vegetative growth of the plants could be significantly and positively correlated with percentage phloem, and—to a less extent—with percentage xylem, while fruitfulness could not be correlated with percentage phloem but was significantly and positively correlated with percentage xylem.

Discussion

The results of two experiments will be discussed. The first involved anatomical studies of tomato stems from plants supplied with nutrient solutions varying in the relative supply of nitrate, sulphate, and phosphate (6); the present experiment involves variations in the relative supply of calcium, magnesium, and potassium. Certain general conclusions seem warranted.

Large variations in the diameter of stem sections from the middle internode of tomato plants can be associated with differences in macronutrient ion supply to the plant. Increased stem diameter can be directly and closely correlated with increased vegetative growth in all cases. Differences in stem diameter were also directly correlated with fruitfulness in the first experiment, in which differences in the fruitful condition of the plant were the result of variations in the anion supply. In that experiment (6), increased nitrate supply was associated with increased fruit production. It is probable that the direct correlation would not hold if levels of nitrate supply sufficiently high to inhibit fruiting had been used. In the second experiment, variations in stem diameter could not be associated in any significant manner with variations in fruitfulness of the plants as a result of altered calcium, magnesium, or potassium supply.

Increased stem diameter and hence cross-sectional area of stem sections was associated with increases in the area of each of the constituent tissue systems. However, variations in the percentage cross-sectional area of each constituent tissue system could also be associated with differences in nutrient composition. Percentage cortex was relatively constant, although small but significant variations occurred in the cation triangle. Differences in the relative area of the vascular tissues could be correlated primarily with the level of potassium, calcium, or nitrate supply. Large differences in the percentage pith occurred between treatments, and percentage pith was inversely correlated with the relative amount of vascular tissue in all cases. Both percentage phloem and percentage xylem were positively and significantly correlated with vegetative growth in both triangles, but correlations involving percentage phloem were invariably significantly higher than those

involving percentage xylem. On the other hand, percentage phloem is not necessarily correlated with fruit production, although significant correlations may be obtained if variations in fruitfulness are the result of differences in nitrate supply. Percentage xylem has invariably been positively and significantly correlated with fruitfulness.

Large differences in cellular characteristics have been correlated with nutrient composition, although more experimentation seems desirable to associate the differences more closely with individual ions. These observations, however, can serve as a basis for future experimentation. For instance, such correlations as calcium ion supply with the development and cellular characteristics of collenchyma; magnesium with chlorenchyma; calcium and nitrate with pericyclic fibers; potassium, calcium, or nitrate with external phloem; nitrate with xylem; and magnesium, potassium, or nitrate supply as they can be correlated with size and structure of internal phloem strands might be useful in evaluating the nutritional status of a tomato plant.

Several anatomical considerations may be of interest. It is evident that both cell size and thickness of cell walls can be related to nutrient composition in so far as the supply of macronutrient ions is varied. With respect to such correlations involving nitrogen, the carbohydrate metabolism of the plant would seem to offer the most logical postulate if a cause-and-effect relationship exists. However, the supply of calcium ion is clearly correlated with cell size and the thickening of cell walls in collenchyma and pericycle. All treatments in the cation triangle were supplied with equal amounts of nitrate in the nutrient medium and contained equal amounts of nitrogen per unit dry weight of leaf at maturity (5). Although plants supplied with relatively large amounts of calcium made less vegetative growth, fruitfulness was materially greater than in plants supplied with relatively low amounts of calcium (4). By computation, it is evident that low-calcium plants produced less total dry weight (roots+vines+fruit) than did high-calcium plants. It seems possible that if carbohydrate utilization is a factor in the anion triangle which can be correlated with cell size and thickness of cell walls, it can also be a factor in the anion triangle. In the anion triangle, different carbohydrate-nitrogen balances may have been attained by a differential in the assimilation of nitrogen. In the cation triangle, a similar difference in the balance may have been attained by a differential in the assimilation of carbohydrate associated with altered relative cation supply.

It seems possible that the results presented here may serve as a guide for future work designed to study the influence of macronutrient elements on cellular physiology. The fact that the supply of a given element may influence the type and amount of differentiation in cell growth may indicate a clue as to the utilization of this element in the cellular metabolism. It seems possible that the plants which received a high calcium supply were more fruitful than those which received a low calcium supply because these plants developed greater amounts of phloem. On the other hand, it is just as possible that the influence of calcium is an indirect one on the utilization of carbohydrate and nitrogenous food material and that the tissue developments correlated with calcium are secondary and the result of the influence of the greater fruitfulness on these tis-

sues. It is possible that results of similar experiments may aid in a clearer understanding of the role of some of the macronutrients in cellular metabolism and differentiation.

Summary

1. An inbred strain of Bonny Best tomatoes was grown in sand culture. The effects of forty-three nutrient solutions varying in the relative proportions of macronutrient cations (calcium, potassium, and magnesium) were studied in relation to the anatomy of plant stems. Measurements of stem diameter and the actual area of each of the component tissue systems were recorded. The data were reduced and analyzed by statistical methods.

2. Great differences in stem diameter and the actual area of each of the component tissue systems were positively correlated with potassium and negatively correlated with calcium supply to the plant.

3. Significant differences between treatments occurred with respect to the relative areas of the constituent tissue systems in the stem sections. These differences could also be correlated with nutrient composition.

4. Cell size and relative thickness of cell walls in pericyclic fibers could be associated with calcium ion supply. Cellular differences were also observed in pith parenchyma, internal and external pericyclic fibers, internal and external phloem, and in cortical chlorenchyma and collenchyma. They are described and correlated with the supply of one or more ions in the nutrient medium.

5. Differences in the anatomy of the tomato stems are significantly correlated with characteristics used as criteria of vegetative growth and fruitfulness. For instance, the relative amount of phloem in stem sections is positively correlated with vegetative growth, while the relative amount of xylem is positively correlated with fruitfulness.

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MORPHOLOGICAL IDENTITY OF THE VELAMEN AND EXODERMIS IN ORCHIDS

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Literature review

The velamen, a specialized tissue of from one to many layers of cells covering the aerial and terrestrial roots of orchids, is separated from the cortical parenchyma by a uniseriate layer of cells which is neither the velamen nor the cortex in structure. This layer, termed endodermis, exodermis, or hypodermis by previous investigators, is composed of vertically alternately long and short cells which may be variously modified structurally and functionally.

SCHLEIDEN (11), who in 1849 named the ensheathing tissue *velamen radicum*, interpreted it as a many-layered root envelope overlying the epidermis. CHATIN (1) also held this opinion. SCHACHT (10) and OUDEMANS (9) thought that the outermost layer of cells was epidermis and that immediately below this was one component of cortex separated from the inner component by a specialized layer, the endodermis (now exodermis). LEITGEB (7) held that the velamen is developed "subsequently and directly from the epidermis by division of its cells." TREUB (13), MEINECKE (8), DE BARY (2), and HABERLANDT (4) adopted LEITGEB's concept and terminology. More recently, JEFFREY (6) and SOLEREDER and MEYER (12) referred to the velamen as a many-layered epidermis.

TREUB and DE BARY seem to be the only ones to have investigated the histogenetic origin of the velamen. The former noted in *Vanilla* and *Stanhopea* that the dermatogen which gave rise to the velamen was developed at the apex from a common initial group for the root cap and the body of the root. DE BARY found a similar situation in *Vanda furva*, but in *Oncidium* he found that "the dermatogen passes over the growing-point as a distinct layer between periblem and calyptrogen."

The textbook of anatomy currently used (3) mentions the velamen of orchids but makes no reference to its morphological identity.

The inner limiting layer of the velamen, termed endodermis by the early investigators, had a special significance to DE BARY, who referred to it as a special case of endodermis. (The latter term he applied to the tissue, adjacent to pericycle, which previously had been called "protective sheath.") HABERLANDT later referred to this endodermis as exodermis.

HAYWARD (5) records for *Linum* a periblem consisting of two layers, the outer one of which divides anticlinally to form the outermost layer of cortex or hypodermis; the inner one divides in all planes to form the remainder of the cortex. Hypodermis is thus made synonymous with exodermis.

It is apparent that several aspects of the problem of velamen ontogeny need investigation. These are (*a*) the histogenetic constitution of the root apex; (*b*) the origin of the velamen—its morphological identity; (*c*) the origin of the exodermis—its morphological identity; (*d*) the relationship between exodermis and hypodermis.

Methods

Aerial and subterranean roots were killed, fixed, sectioned in paraffin, and stained with safranin or with Delafield's haematoxylin.[1] No counterstains were used. The roots of twenty species and hybrids, representing thirteen genera of epiphytic and terrestrial orchids, were

[1] Thanks are due MAMORU ISHII for preparing more than half the slides.

investigated. These, with the number of rows of cells in the velamen, are as follows:

Aerides lawrenceae Rchb. f.*	4 rows
Arundina graminifolia Scltr. (*A. bambusifolia* Lindl.) [terrestrial]†	2
Brassavola cucullata R. Br.†	5–6
Brassavola nodosa Lindl.†	5–7
Brassia brachiata Lindl.†	10–12
Cattleya manoa†	5
Cattleya percivalliana Rchb. f. × *C. santa monica*†	4–5
Cattleya trianae Rchb. f.†	8–10
Dendrobium sp.	3–4
Epidendrum catillus Rchb. f. et Warse†	6
Epidendrum cinnabarinum Salzm. [terrestrial]‡	6
Epidendrum radicans Pav.†	6
Oncidium sphacelatum Lindl.†	8–10
Phalaenopsis schilleriana Rchb. f.†	3
Phalaenopsis sumabilis (*P. sumatrana* Korth. × *P. amabilis* Bl.)†	2
Spathoglottis × *parsonii* × *S. plicata* Bl. [terrestrial]†	2
Sobralia macrantha Lindl. [terrestrial]	3–4
Vanda luzonica Loher†	6
Vanda parishii Rchb. f. (*Vandopsis parishii* Schltr.)†	7–8
Vanilla planifolia Andr.*	1

* Obtained from the Foster Gardens through the courtesy of COLIN POTTER.

† Obtained from plants in the Frank Atherton collection through the courtesy of O. M. KIRSCH.

‡ Obtained through the courtesy of Dr. C. P. SIDERIS of the Pineapple Research Institute. All of Honolulu.

Investigation

HISTOGENETIC CONSTITUTION OF APEX

Certain of the histogen types established by HANSTEIN and developed by JANCZEWSKI and HABERLANDT, although no longer applicable to the stem apex, will be utilized in this study. The root tips of all but six of the twenty species investigated belong to type I, in which four distinct histogens give rise to all tissues of the root. The remaining six—*Aerides lawrenceae, Phalaenopsis sumabilis, P. schilleriana, Vanda luzonica, V. parishii,* and *Vanilla planifolia*—belong to HABERLANDT's type VI, in which the tissues are derived from a common mass of cells at the root apex but in which the protoderm takes no part in root-cap formation as in type IV.

Figure 1, a portion of the root apex of *Sobralia macrantha,* is representative of the others of this histogen category. The cells of the histogens, the protoderm and its derivatives, the exodermis, and the peripheral cells of the procambium have been stippled for emphasis. The emphasis is not unduly exaggerated, for these tissues show up clearly with the single-stain procedure (fig. 3). Figure 2, *Phalaenopsis sumabilis,* shows the cells of the protoderm and its derivatives, the exodermis. There is not one distinct histogen in this root; the fundamental tissues arise from a large mass of meristematic cells. In other roots of this type there is developed a distinct calyptrogen below the embryonic mass and just outside the protoderm. The latter tissue does not contribute to the calyptra or calyptrogen.

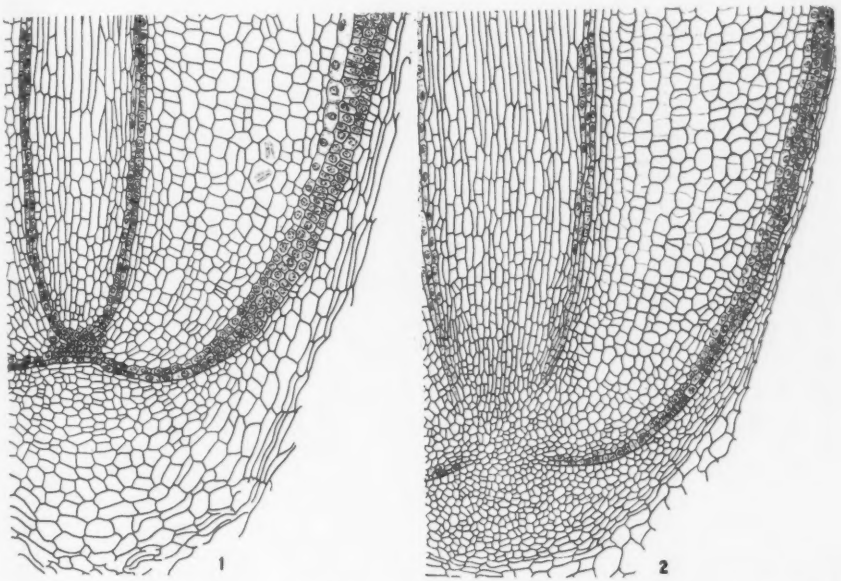
These two types of histogenetic constitution were observed by TREUB in *Vanilla* and *Stanhopea* (type VI), and by DE BARY in *Vanda furva* (type VI) and in *Oncidium* (type I). On the basis of results of the present study, apparently roots with a distinct dermatogen are the more common in orchids.

ONTOGENY OF VELAMEN

The velamen is derived from the protoderm by periclinal divisions (figs. 1, 2, 4, 5). The cells of the latter, which are alternately long and short (fig. 5), become vacuolated early, and they thereby clearly mark the internal boundary of the velamen. In *Vanilla* the velamen is only one cell in width; in *Oncidium* it is

8–10 cells in width. Root hairs are frequently developed (fig. 7).

Although there appears in the literature no general agreement of definition of epidermis, the term multiple or many-layered epidermis has often been used. Some investigators seem to prefer to limit the concept of epidermis to the outermost layer of tissue. Since in the ontogeny of the many-layered dermal system the cells are derivatives of the protoderm, they are therefore closely related histologically. The velamen is here considered *in toto* to be multiple epidermis.



FIGS. 1, 2.—Fig. 1, camera lucida diagram of portion of root tip of *Sobralia macrantha* showing type I histogens and origin of velamen and exodermis. Fig. 2, same of *Phalaenopsis sumabilis* showing type VI histogens and origin of velamen and exodermis.

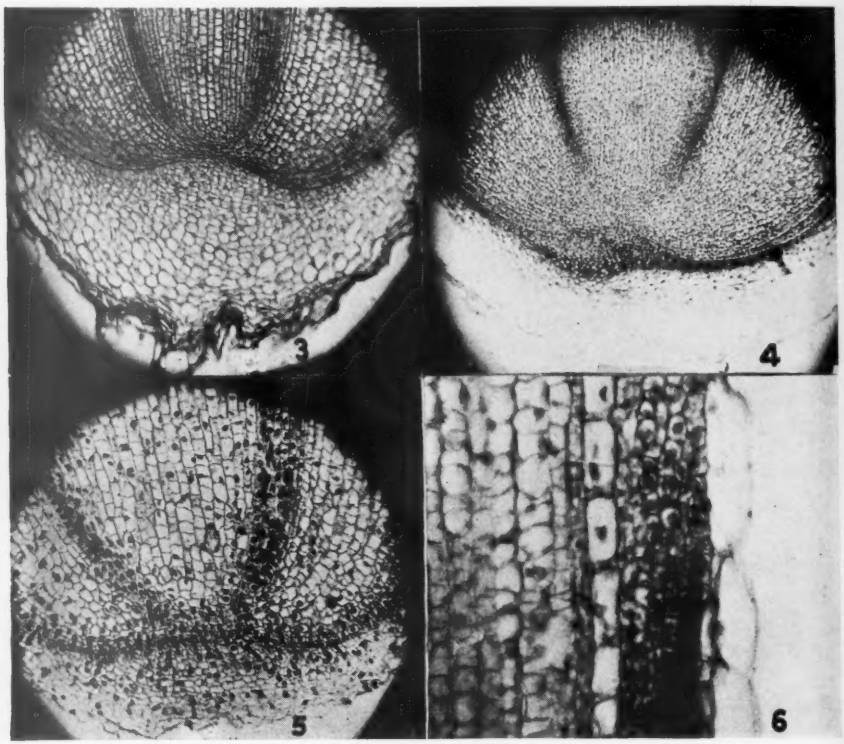
ORIGIN OF EXODERMIS

It was noted previously that the layer of cells forming the inner limit of the velamen has been considered to be (*a*) the endodermis proper, and (*b*) a special kind of endodermis called exodermis. In all roots investigated, this limiting tissue is derived from the periblem and is therefore to be considered morphologically as specialized cortical tissue (figs. 1, 2).

Owing to the lack of discrete histogens in the type VI root tips, it is impossible to establish a histogenetic origin for the exodermis in those roots. The exodermis is clearly discernible as a distinct uniseriate row of cells all the way down to the meristematic group. It is in all respects similar in its ontogeny to its counterpart in type I roots, and it should be considered at maturity as the specialized outermost layer of the cortex. The term exodermis is retained in this paper as proper terminology to apply to this tissue, since this tissue is similar to the endodermis in its ontogeny and in its

outer limiting position in the cortical tissue.

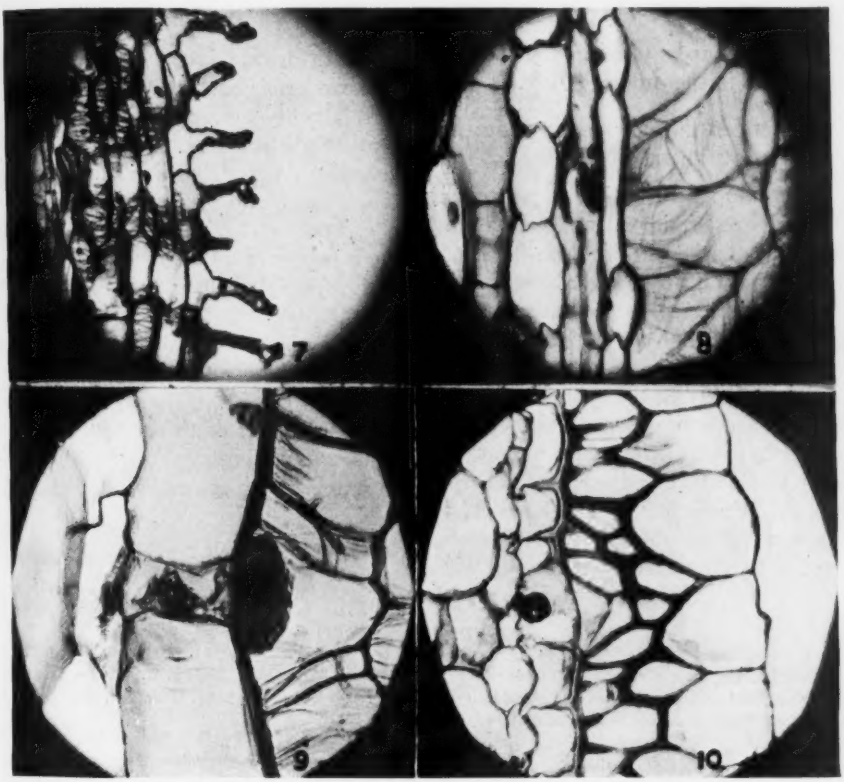
The structure of the mature exodermis is much more specialized than that of the endodermis. Early in the ontogeny of the exodermis, vertical rows of cells are alternately elongate and short (fig. 6). In the early investigations these were called long endodermis cells (*lange Endodermiszellen*) and short endodermis cells (*kleine Endodermiszellen*); later the short cells were termed passage cells, similar to their counterparts in the endodermis of certain roots. The long cells develop thick secondary walls on the radial, and especially on the outer tangential, walls. The passage cells remain thin-walled. The protoplasm of the latter cells is dense, and the nucleus is very large and prominent; but in the long cells the protoplasm is sparse and the nuclei less prominent.



FIGS. 3–6.—Fig. 3, root tip of *Sobralia macrantha*. Fig. 4, root tip of *Vanda luzonica*. Fig. 5, root tip of *Epidendrum cinnabarinum*. Fig. 6, longitudinal view of developing velamen (at right) and prominent exodermis showing long and short cells.

The cells of the velamen are variously thickened, but those adjacent to the passage cells fall, according to the species, into one of several types. In the simplest kind the velamen cells next to the passage cells are similar to the other cells of the velamen, as in *Brassia brachiata* (fig. 8); there is no specialization. In

species of *Cattleya*, the cells adjacent to the passage cells, as in *Brassia*, are not thickened, or are only slightly thickened, as in *Cattleya trianae* (not here illustrated). The most highly developed velamen cells adjacent to passage cells are found in *Sobralia macrantha* (fig. 9). These have been depicted diagrammatically by MEINECKE (8) and by HABERLANDT (4). A large hemispherical pad of fibrous material develops on the inner tangential walls of the velamen cell (or cells) adjacent to the passage cell. This was termed by MEINECKE *Stabkörper*, or fibrous body, and that name is still used. The body is composed of very minute fibrous ridges and rods which become enmeshed. In certain orchids, especially those with a velamen comprised of only a few layers of cells, the thin-walled passage cell is covered by a group of wedge-shaped cells, the walls of which are much thinner than adjacent velamen cells (fig. 10, *Dendrobium*). HABERLANDT discusses the structure of certain aerial roots, among them orchids. In *Taeniophyllum zollingeri* he has described a structure comprised of three



FIGS. 7–10.—Fig. 7, portion of root-hair group developed from outermost velamen cells in *Vanda luzonica*. Fig. 8, portion of exodermis showing thin-walled passage cells and velamen of *Brassia brachiata*. Fig. 9, passage cell and fibrous body of *Sobralia macrantha*. Fig. 10, thin-walled passage cells of *Dendrobium* sp. showing cover cells in velamen.

distinct parts: a mass of velamen cells attached to a single or to two or three thin-walled, air-containing exodermal cells which in their turn abut one or more rows of special rounded parenchymatous elements, the latter of cortex. This apparatus he refers to as a pneumathode. None were encountered in the roots investigated in the present study.

Relationship between exodermis and hypodermis

It was noted earlier in this paper that the term exodermis replaced endodermis, and that the latter term was then applied to the inner limiting layer of cortex. Hayward referred to the outer limiting layer (exodermis) as hypodermis. De Bary, and later Eames and MacDaniels, described the hypodermis as essentially sheets or groups of tissue, often sclerenchyma, which form a supporting or protective layer adjacent to the epidermis. Hypodermis thus defined refers to spacial relationship with the epidermis and not to ontogenetic relationship with the cortex, and could more appropriately be referred to as cortical sclerenchyma, etc. The exodermis should not be considered in the same category as hypodermis.

Summary

1. Historical concepts of the velamen and exodermis are reviewed.

2. Twenty species and hybrids, representing thirteen genera of orchids, were investigated to determine the origins of the velamen and exodermis.

3. Fourteen orchid root tips belong to the category of histogens known as type I; the other six belong to Haberlandt's type VI.

4. The velamen is derived from the protoderm. It is a multiple epidermis.

5. The exodermis is the specialized outermost layer of the cortex. It corresponds, in its limiting position and similar ontogeny, to the endodermis.

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NATURE AND RATE OF DEVELOPMENT OF ROOT SYSTEM OF APOCYNUM CANNABINUM[1]

JOHN C. FRAZIER

Introduction

This is the fourth of a series of reports on growth habits of noxious perennial weeds of central United States being conducted by the Kansas Agricultural Experiment Station. A study (2) of the nature and rate of development of field bindweed established the scope and methods of procedure. The second investigation (3) dealt with hoary cress, and the third (4) with Russian knapweed. The present study is concerned with dogbane, *Apocynum cannabinum* L., a noxious weed of potential significance rivaling that of field bindweed, hoary cress, and Russian knapweed, particularly when its widespread distribution and its abundance are considered. Its noxiousness is not usually appreciated. GATES (5) states that it is one of the weeds of statewide distribution which are increasing markedly in abundance in Kansas.

Environmental conditions and methods

SOIL DATA.—All plants[2] considered in this paper were taken from soil, the profile for the upper 60 inches of which was described by FRAZIER (3). The lower 24 inches of this 7-foot profile, which was mellow throughout, had the same structure as the 22–60-inch interval. The entire profile description was supplied by Dr. J. C. HIDE of the Kansas Agricultural Experiment Station.

METEOROLOGICAL DATA.—The monthly and annual temperature and precipitation data for the first 10 months of 1943 compared with the long-time average of these factors are given in table 1. The summer of 1943 had temperatures of 100° F. or higher 9 days as compared with 38 such days in 1937, when the comparable study was made on field bindweed; 15 in 1941, when the comparable study was made on hoary cress; and 5 in 1942, when the comparable study was made on Russian knapweed. The average annual number of days having temperatures of 100° F. or higher during the 50 years, 1889–1938, is 15. The average monthly precipitation for the first 10 months of 1943 was 3.18 inches, as compared with the monthly average of 2.86 for these months during the 83-year period, 1858–1940. Some of the precipitation for the first 10 months of 1943 fell as heavy rains. There were three 24-hour periods in June with 2.93,

TABLE 1

MONTHLY AND ANNUAL TEMPERATURES (IN ° F.) AND PRECIPITATION (IN INCHES) AT MANHATTAN, KANSAS, 1943

MONTH	TEMPERATURE		PRECIPITATION	
	Mean*	85-year average (1856–1940)†	Total*	83-year average (1858–1940)†
Jan.	27.8	27.0	0.22	0.75
Feb.	39.6	31.5	1.15	1.14
Mar.	40.0	42.5	0.98	1.48
Apr.	58.6	54.5	1.15	2.67
May	62.0	64.6	3.14	4.31
June	75.7	74.5	12.28	4.50
July	79.5	79.5	4.68	4.25
Aug.	83.4	77.5	1.71	3.89
Sept.	67.6	69.3	4.23	3.44
Oct.	56.6	56.5	2.29	2.16
Annual mean or total	55.2	54.2	34.13	30.97

* Meteorological data obtained from U.S. Weather Bureau, Kansas section.

† Computed by Dr. A. B. CARDWELL of Kansas State College.

[1] Contribution no. 455, Department of Botany, Kansas Agricultural Experiment Station.

[2] The term plant as used in this paper refers to all the growth from a single seed. The term shoot is applied to the individual leafy stems commonly called plants in control studies.

FIGS. 1–3.—Stages in development of plants of dogbane: Fig. 1, seedling 2 weeks after emergence showing first pair of true leaves above the two cotyledons; *F*, root-stem transition zone. Scale in inches. Fig. 2, young plant 10 weeks after seedling emergence showing relation of primary vertical root (*P*) to permanent lateral roots (*L*) of first order (growing tips not obtained). Scale in feet and inches. Fig. 3, piece of permanent lateral root bearing short rhizome; latter had grown approximately one-third of distance to soil surface. Note scale leaves on rhizome.

2.65, and 1.82 inches, respectively, one in July with 1.48 inches, and one in September with 1.52 inches.

METHODS.—The plants were grown on a small plot of land free of other noxious weeds and of sodium chlorate. The soil is a fairly typical Geary silt loam representative of the wind-deposited soil of the upland of this locality. The plot had been cultivated a decade earlier but had not been disturbed recently.

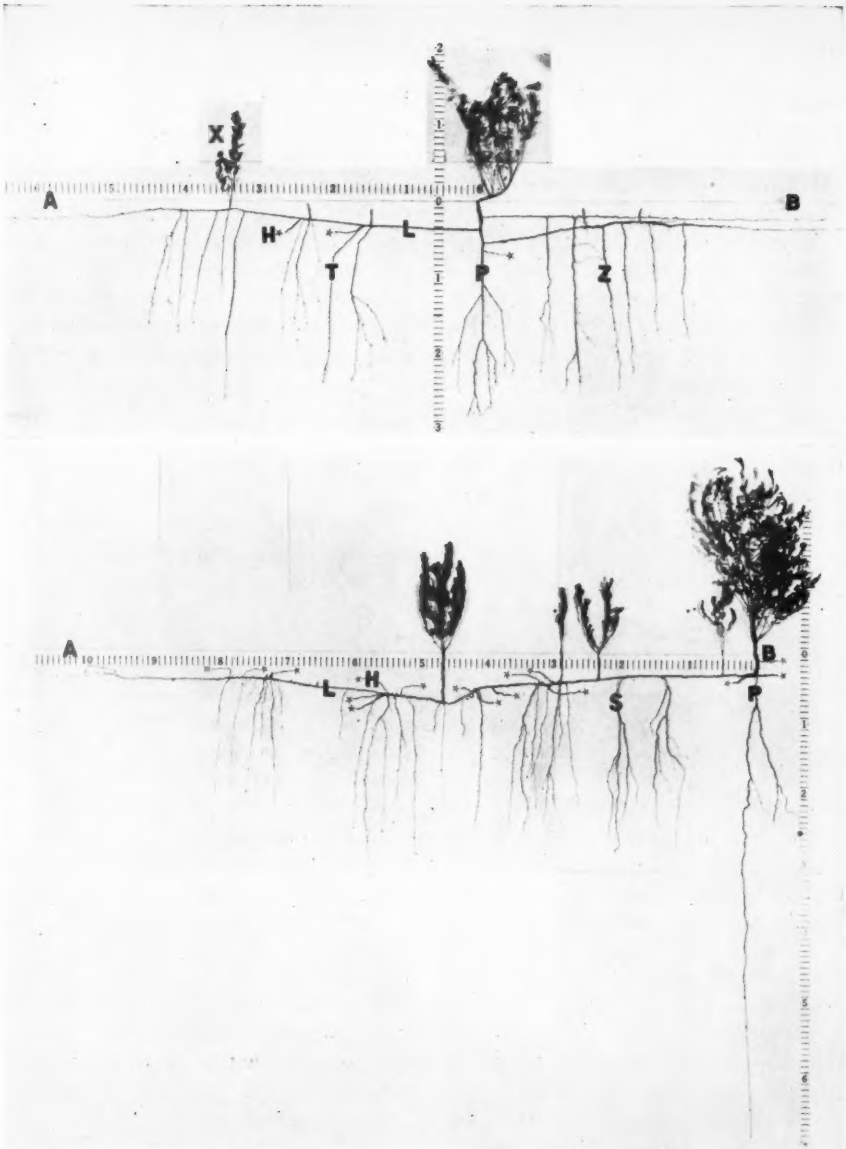
The first planting was made on April 2, 1943. Three seedlings emerged from this planting, but only the one which emerged on April 15 lived. It was excavated after 7 months of growth and was designated plant no. 8. A second planting was made on May 3 and emergence began May 14. On May 17 seven seedlings emerged. The plant developing from one was excavated after 5 months of growth. It was designated plant no. 7. A third planting was made on May 15. Emergence began May 26, and on May 27, eleven seedlings emerged at as many points. Plants developing from six of these were used for plants 1–6, inclusive. In each planting, twenty seeds, which had been kept moist at 34° F. for at least 14 days, were placed $\frac{1}{4}$ inch below the surface at each of eighteen points which were so spaced that eight of the plantings were 15 feet distant from any other and the remainder were separated by 9 feet. All the nineteen seedlings emerging on the three dates (April 15, May 17, and May 27) were retained, but all others, including those emerging after May 27, were removed. The area was kept free of all other vegetation, so that the only competition for water and plant nutrients, if any existed, was among the plants of dogbane.

The root systems were excavated by modification of the trenching method developed by WEAVER (10) as described by FRAZIER (2). Efforts were made to keep the root system as one organic entity; if a root broke, the two ends were immediately tied together. Measurements of the plant parts were made and recorded, so that as illustrated they occupied the same relationship, one part to another, as they did in the soil, except that in figures 1, 2, 4, and 5 all lateral roots are arranged in one plane. While as many as possible of the finer roots were obtained, it is not contended that a large portion of them was secured.

Observations

The rate of plant development under known soil and climatic conditions was observed by means of eight plants excavated 1, 2, 4, 5, 8, 10, 20, and 28 weeks after seedling emergence. The dates of excavation were June 3, June 10, June 24, July 1, July 22, August 5, in early October, and in early November, 1943. The plants are designated 1–8, inclusive. Figures 1, 2, 4, and 5 show the vertical penetration and the radial spread of roots of representative plants. Data of the eight excavations are given in table 2.

By 10 weeks after emergence the general plan of the root system was becoming established (fig. 2). Plant no. 7, taken the twentieth week after emergence (fig. 4), showed particularly well the gross morphological nature of the root system. A taproot rapidly penetrated directly downward from the germinating seed. It was a primary root in order of development and vertical in position; hence, it is designated the primary vertical root (fig. 4*P*). Many branch roots arose throughout the length of this taproot, most of them small feeding roots. A few, probably those more favorably situated in relation to soil moisture and plant nutrients, grew extensively and became per-



FIGS. 4, 5.—Plant parts arranged to show essentially same relationship as in the soil. *A-B*, ground line. Scales in feet and inches. Fig. 4 (above), complete plant 20 weeks after seedling emergence: *P*, primary vertical root; *L*, permanent lateral root of first order (star at right of center indicates where permanent lateral root of first order was removed for clarity); *H*, permanent lateral root of second order (two stars at left indicate permanent laterals of second order removed); *Z*, secondary vertical root which arose on lower side of permanent lateral root and grew downward; and *T*, secondary vertical root which arose on side of permanent lateral root and grew horizontally approximately 7 inches before turning downward. Note large root system produced by the two shoots, smaller of which emerged only 5 weeks earlier. Fig. 5 (below), portion of plant 28 weeks after seedling emergence. Primary vertical root (*P*) subtending extensively developed original shoot; stubs of three permanent lateral roots of first order indicated by stars immediately to right and left of primary vertical root at ground line; and approximately 70% of root and all of shoot development of longest, typical, permanent lateral root of first order (*L*) showing the four rhizomes, each bearing a shoot, the twenty-nine secondary vertical roots (*S*), and stubs of thirteen permanent lateral roots of second order (*H*), each designated by star indicating a root removed.

manent laterals (fig. 4*L*). They are designated permanent lateral roots of the first order. These roots tended to grow as radii about the primary vertical root in a plane parallel to the soil surface in the upper foot, more commonly in the upper 7 inches of the soil. They gave rise to branch roots over any part of their growth. Most of these were small feeding roots. However, some arose which appeared similar to these feeding roots. They developed into branch lateral roots, which are designated permanent lateral roots of the second order (figs. 4*H*, 5*H*). They grew horizontally at right angles to the root on which they arose for a distance of approximately 2–8 inches, then the majority grew horizontally away from the primary vertical root. In a similar manner permanent lateral roots of the third order arose on those of the second, and those of the fourth order on those of the third, etc. This branching tended to develop in the area between the permanent laterals of the first order. The result was that the plant occupied a much higher proportion of the ever-increasing area, somewhat circular in shape, than it would have done otherwise. The permanent laterals of the first order outgrew those of the other orders, however, so that they extended a greater horizontal distance from the primary vertical root. Concurrent studies indicated that plants subject to severe competition made less rapid horizontal development.

In addition to the primary vertical root (figs. 4*P*, 5*P*), other vertical roots were formed. These branch roots sometimes arose on the lower side of the permanent lateral root of any order, in which

TABLE 2

RATE AND NATURE OF DEVELOPMENT,* AFTER SEEDLING EMERGENCE, OF ROOTS OF DOGBANE PLANTS. MANHATTAN, KANSAS, 1943

Plant no.	Weeks after emergence	Maximum vertical penetration (inches)	Maximum radial spread (inches)	Plant condition
1	1	$1\frac{3}{4}$	$\frac{1}{4}$	Typically shaped cotyledons (fig. 1)
2	2	$2\frac{1}{2}$	$\frac{3}{8}$	First true leaves (fig. 1)
3	4	$6\frac{1}{8}$	$2\frac{1}{2}$	Additional true leaves, primary stem axis beginning to elongate
4	5	$9\frac{3}{4}$	$4\frac{1}{8}$	Cotyledons present on this plant, lost from others of same age
5	8	$21\frac{1}{4}$	$10\frac{3}{4}$	Primary stem axis 5 inches long; secondary stem axes developing; cotyledons lost
6	10	$28\frac{3}{4}$	Approx. 20–24	Secondary stem axes well developed (fig. 2)
7	20	$34\frac{1}{2}$	$76\frac{3}{4}$	Lateral roots of first order have produced branch lateral roots (those of second order); vertical roots (secondary verticals); and many root-borne stem buds, some of which have produced rhizomes (fig. 3). A few of the latter have produced shoots (fig. 4X)
8	28	6′11½″	11′8½″	Primary vertical root had penetrated twice the depth attained by any secondary vertical. Lateral root of first order extending greatest distance from primary vertical root had produced 13 lateral roots of second order, 29 secondary vertical roots, and 4 rhizomes which had produced shoots (fig. 5)

*First flowering occurred May 25, 1944.

case they grew directly downward (fig. 4*Z*). More commonly they arose on the sides or top of the permanent lateral roots of any order, in which case they grew horizontally approximately 2–12 inches and then bent downward to become vertical taproots (fig. 4*T*). Both types are designated secondary vertical roots. While these vertical roots arose on permanent laterals of any order, they naturally arose first on permanent lateral roots of the earlier formed orders.

All shoot development, except that from the plumule, was derived from root-borne stem buds. In the one growing season of 28 weeks, seven root-borne buds were produced on permanent laterals of the third order, twelve on those of the second order, and at least twenty-seven on those of the first order. None was produced on the primary or secondary vertical roots.

By 15 weeks after seedling emergence, a number of these buds had formed on the permanent lateral roots of the first order about 20 inches from the primary vertical root. Twenty weeks after seedling emergence such root-borne buds had produced rhizomes (fig. 3), some of which had grown to a length of 4 inches, and one upon reaching the surface had developed a shoot (fig. 4*X*). These root-borne stem buds gave rise to rhizomes unless they were borne at the ground line, in which case they gave rise to leafy shoots.

Twenty-eight weeks after seedling emergence, a plant (fig. 5) had sent its primary vertical root to a depth of 6 feet 11½ inches and had produced four permanent lateral roots of the first order which were arranged radially around the primary vertical root. Three of these permanent laterals of the first order had formed lateral roots of the second and higher orders. One was extensively developed in regard to the number of permanent lateral roots of the second and higher orders, and in total length. The extent of the total growth in length was 16 feet 2 inches, but this particular permanent lateral, unlike all others observed, did not grow as a radius from the primary vertical root; it extended but 10 feet 3 inches from that root. Another attained a length of 11 feet 8½ inches from the primary vertical root. This permanent lateral root is shown in figure 5 in the same relation it had in the soil to its secondary vertical roots, the permanent lateral roots of the second order, and the primary vertical root. This figure also shows the relation of this permanent lateral of the first order to its four shoots. There were sixteen shoots on the plant—the larger one borne above the primary vertical root, four on the permanent lateral shown in figure 5, and eleven on the more extensively developed lateral just mentioned. The two permanent lateral roots of the first order, which were much less extensively developed, had no shoot development. The growth attained 28 weeks after emergence was so carefully removed that at least 90% of the plant was recovered. From it the material shown in figure 5 was obtained.

Discussion

The rate of growth of plants of dogbane from seed under known soil and climatic conditions, with little or no competition, is not to be construed as representing their growth rate under all conditions. It illustrates only the growth potential in a favorable noncompetitive situation.

Observations made in this investigation agree with previous studies on this plant. HITCHCOCK and NORTON (7) studied the seedling stage. Their sketch is similar to the shoot development of a

seedling 2 weeks after emergence (fig. 1), but it does not show the root so extensively developed. The deep penetration of the vertical roots was noted years ago by HITCHCOCK and NORTON (8). In the present study vertical penetration by the primary vertical root was approximately 7 feet in a growing season of 7 months.

The nature and extent of the horizontal growth of the underground lateral axes, the recognition of these axes as roots rather than as stems, and the relation of the rhizome to the horizontal roots and to the shoots were clearly shown by HITCHCOCK and CLOTHIER (6) in 1898. They observed, as was confirmed in the study here reported, that there was no regularity of the placing of these root-borne buds on the lateral roots. CUNNINGHAM (1) also considered the horizontal axes as roots and called attention to their being commonly located relatively close to the surface.

Research to determine why certain of the branch roots develop more extensively than others and become permanent lateral roots has not been undertaken. It has been conjectured by KENNEDY and CRAFTS (9) for field bindweed and by FRAZIER (3) for hoary cress and (4) for Russian knapweed that these variations result from differences in the supplies of soil moisture and plant nutrients.

Marked contrast exists in the nature of the development made by the permanent lateral roots of the first order in dogbane as compared with the other three plants considered in this series (2, 3, 4). In the dogbane the lateral roots of the first order grew horizontally throughout the season. In no instance were the growing tips of these lateral roots observed to turn downward and thus become vertical roots. One such lateral root attained a total length of 16 feet 2 inches in a growing season of 7 months. HITCHCOCK and CLOTHIER (6) reported a horizontal root, not traced to its end, which was 29 feet in length. They also observed the same type of branching found in this study, whereby lateral roots of the second order arose on those of the first order.

Summary

1. Plants of dogbane, grown from seed on a typical upland loam soil at Manhattan, Kansas, under known temperature and precipitation conditions and not subject to competition, were studied at various ages, from the seedling stage through 28 weeks of growth, to determine the nature and rate of development.

2. The root system of well-established plants consisted of the original root (primary vertical) and one to many permanent lateral roots which continued to grow horizontally and on which arose roots that either grew downward directly, or did so after short horizontal growth, to become secondary vertical roots.

3. The plants spread horizontally by means of these permanent lateral roots. The permanent laterals of the first order arose on the primary vertical root. Branch lateral roots (laterals of the second order) arose on the permanent lateral roots of the first order. In a similar manner permanent lateral roots of the third order arose on those of the second, and those of the fourth order on those of the third, etc. Concurrent studies indicated that injury or too severe competition prevents extensive lengthwise growth of the lateral roots of any order.

4. The plants spread radially $11\frac{2}{3}$ feet and reached a depth of 7 feet in a growing season of 28 weeks.

5. The source of shoot development, other than that arising from the plumule, was from root-borne buds which pro-

duced shoots directly (if at the ground line), or rhizomes (if below ground) which in turn gave rise to leafy shoots. There was no regularity to the location of these adventitious buds on the permanent lateral roots. None of these buds was observed on primary or secondary vertical roots. The shoot development of old plants was wholly from root-borne buds.

6. The general type of development of dogbane exhibits certain similarities to, and certain differences from, the type common to field bindweed, hoary cress, and Russian knapweed. The horizontal spread in both types is by means of permanent lateral roots, but in the dogbane type the lateral roots continue to grow horizontally and do not bend downward to produce vertical roots (secondary verticals) as they do in the other type. In the dogbane type, secondary vertical roots arise on the permanent lateral roots. In general, dogbane and field bindweed appear to grow more rapidly, at least in radial spread, for the first growing season of 28 weeks than do hoary cress and Russian knapweed, when all are free from competition. Dogbane had the most rapid vertical penetration in this interval. Dogbane has the coarsest and bindweed the finest roots. The development of shoots appears to be the same in both types. Bindweed was the only one of the four species considered in these studies which flowered during the first growing season.

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SOME TELEMORPHIC EFFECTS INDUCED IN SWEET PEA BY APPLICATION OF 4-CHLOROPHENOXYACETIC ACID[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 560

J. M. BEAL

Several recent papers by ZIMMERMAN (3) and ZIMMERMAN and HITCHCOCK (4) have described the formative influences induced in plants by application of various growth-regulating substances (see 3 and 4 for literature). Because pronounced morphological responses are often induced or incited at considerable distance from the point of application of the substance, the term "telemorphic" effect or response is proposed to include them.

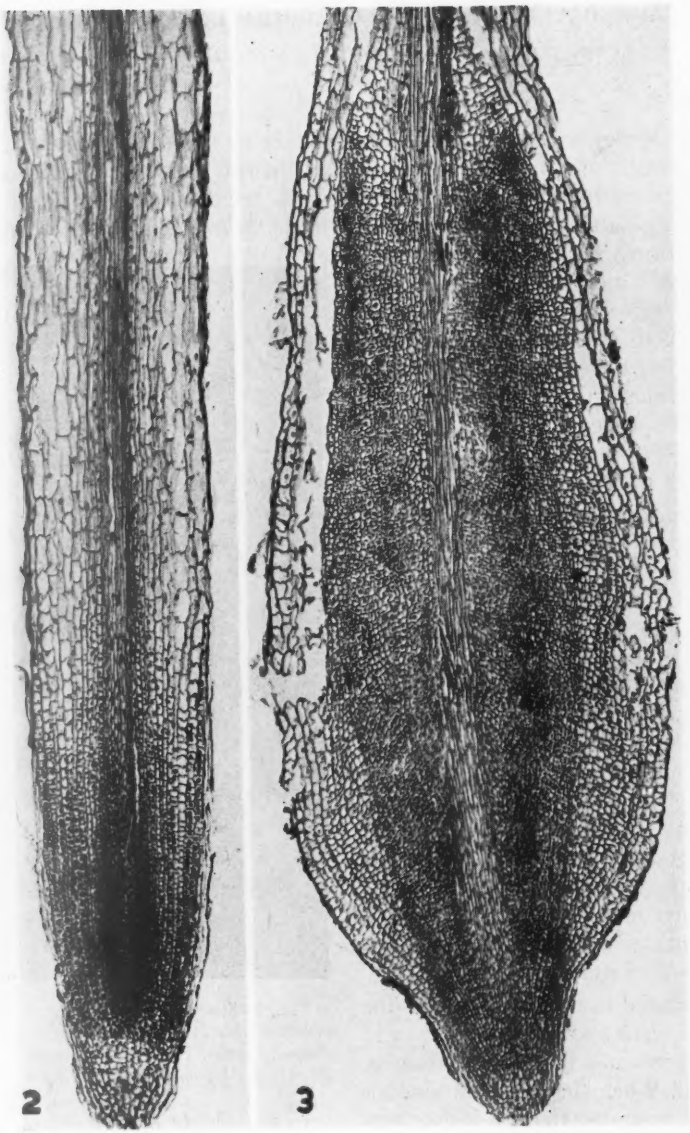
Most of the illustrations and descriptions of such telemorphic effects now recorded show such responses as mainly have been induced in the above-ground portions of treated plants. A few (1) relate them to the roots or root system. MITCHELL and HAMNER (2) have described the use of Carbowax as a carrier for growth-regulating substances—a method for applying precise amounts and for making determinations of the amount of growth made subsequent to application. Although they state that the substances—or derivatives from them—pass through the treated plants in both directions from the point of application, none of their illustrations shows responses or effects in the roots.

The material here illustrated is of the sweet pea (*Lathyrus odoratus*), grown in the greenhouse in 4-inch pots containing fertile soil. When the plants attained a height of approximately 4 inches and produced the first well-developed foliage leaf, a small amount of a 1% mixture of the growth substance was applied—either by smearing a small area of each of the two leaflets or by ringing the stem with the mixture just above this leaf node. Several substances were used.

FIG. 1.—Sweet pea plant 21 days after treatment on leaflet with 4-chlorophenoxyacetic acid at place shown by arrow. Telemorphic effects evident in upper and lower portions of stem, leaves, and roots.

In the plants to which 4-chlorophenoxyacetic acid was applied by either of these methods, evident stem curvature and epinasty followed in a few hours. The terminal bud was decidedly retarded and had elongated comparatively little, even after 4 weeks. The next leaf above

[1] This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of The University of Chicago.



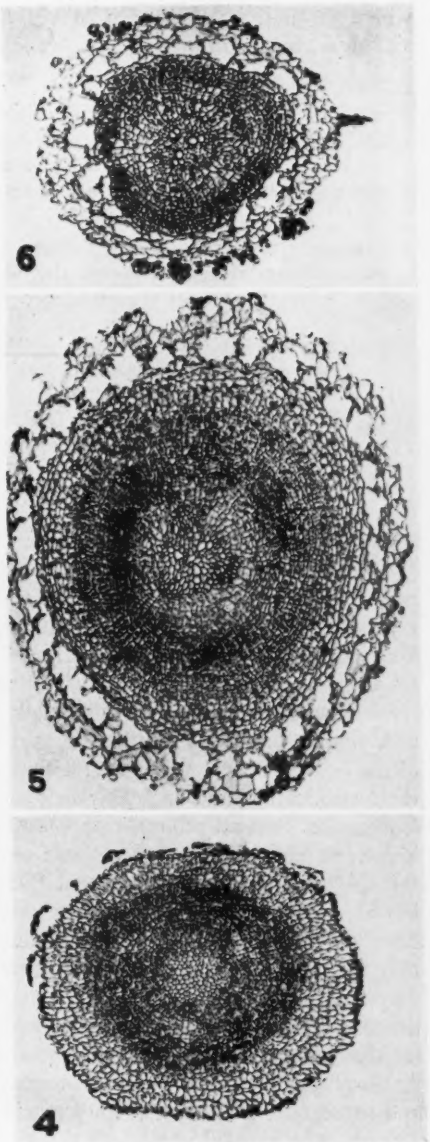
FIGS. 2, 3.—Fig. 2, longisection of root from control plant. Fig. 3, longisection of root from plant treated with 4-chlorophenoxyacetic acid showing marked proliferation of pericycle and some proliferation of endodermis.

the point of treatment developed apparently normally, however, possibly because it was already fairly well formed and partly expanded at the time of treatment. The internode between this leaf and the terminal bud elongated to about normal length, but thickened noticeably and became a light yellowish green color. The terminal bud was decidedly deformed and appeared to have ceased elongation (fig. 1). An even more striking effect occurred in the roots of these plants. When removed from the soil, many of them were thickened for variable distances just back of the apex (fig. 1).

A longitudinal section of a control root is shown in figure 2 and of one of the swollen roots in figure 3. In the latter there is no suggestion of the presence of root tubercle bacteria in any of the cells. The influence of the growth substance has extended to the root tip and appears to have caused some modification of the root cap. The primary xylem and phloem are affected little, if at all, but the pericycle has proliferated greatly (figs. 3–6). There are indications that the endodermis has proliferated to some extent (figs. 4–6).

Sweet peas were treated with several other growth-regulating substances at the same time and in the manner described. The roots of none of these treated plants showed evident enlargement, although a few appeared to have slightly swollen root tips after treatment with α-naphthaleneacetic acid.

Three other kinds of plants, belonging to different genera and (except for the bean) to different families, have been treated in the same manner as the sweet peas. Only one of these, the African marigold, has shown telemorphic effects at or near the ground line. This species was highly sensitive and responsive to most of the chemicals employed. There is con-



FIGS. 4–6.—Transections of root similar to that in fig. 3. Fig. 4, near root apex. Fig. 5, near mid-region. Fig. 6, near proximal portion. Pericyclic proliferation evident with less endodermal activity.

siderable difference in the responses which an individual species makes to similar growth-promoting substances and a tremendous difference between different genera.

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POLYETHYLENE GLYCOLS AS CARRIERS FOR GROWTH-REGULATING SUBSTANCES

JOHN W. MITCHELL[1] AND CHARLES L. HAMNER[2]

Introduction

Growth-regulating substances are not readily effective when applied in the dry crystalline state to the surface of plants. Since most of these compounds are not readily soluble in water, various other carriers and solvents—such as lanolin, oils, or dilute aqueous solutions of ethyl alcohol—have been used to facilitate their application and increase their effectiveness. Several related compounds, known as polyethylene glycols and obtainable under the trade name of Carbowax, are also adapted to this purpose. They differ from lanolin and oils in that they are readily soluble in water, and they differ from aqueous alcoholic solutions in that they are nonvolatile and relatively nontoxic to plants. They readily dissolve growth-regulating substances and serve as efficient spreaders when in aqueous solutions (2).

It was the purpose of this investigation (*a*) to study some of the properties of Carbowax compounds in relation to the application of certain growth-regulating substances to plants, and (*b*) to consider the effectiveness of these wax-like substances in comparison with other carriers and determine quantitatively their influence on the effectiveness of some growth-regulating substances, particularly 2,4-dichlorophenoxyacetic acid.

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Results

PROPERTIES OF CARBOWAX COMPOUNDS.—Three compounds are commercially available—nos. 1500, 1540, and 4000. The first is relatively soft and has a melting point of 34°–37° C.; no. 1540 is somewhat harder and melts at 40°–45° C.; while no. 4000, the hardest, melts above 50° C. Their solubility in water at 20° C. is 62, 59, and 53% by weight, respectively (data supplied by National Carbide and Carbon Corporation). No. 1540 was found to be relatively soluble in acetone, benzene, and 95% ethyl alcohol; less soluble (or insoluble) in xylol, absolute ethyl alcohol, mineral

oil, glycerin, ethylene glycol, turpentine, and in liquid freon and dimethyl ether—all at room temperature. The pH of a 0.5% aqueous solution of Carbowax 1500 was 4.8 as compared with 5.2 for the distilled water alone. Carbowax 1500 and 1540 are hygroscopic, and when placed on a leaf they take up water and appear moist. Under very humid conditions they may become liquid and spread over the leaf surface. Carbowax compounds also have the property of lowering the surface tension of water, the addition of 0.1% by weight of no. 1500 resulting in a solution with a surface tension of 58 dynes, and a 1% solution, 38 dynes, in contrast with water—which has a surface tension of 72.8 dynes at 20° C.

Various growth-regulating substances—including indoleacetic, naphthaleneacetic, naphthoxyacetic, indolebutyric, 2,4-dichlorophenoxyacetic acids, and naphthalene acetamide—proved to be soluble in Carbowax compounds (**2, 3**). By first dissolving the substances in the melted wax, then adding this solution to water, it was possible to prepare aqueous solutions of relatively high concentrations (table 1). A simple method of making aqueous solutions containing different known amounts of a compound consisted of first preparing a concentrated solution of the substance in the melted wax, then weighing out the required amount of this as a solid and dissolving it directly in the required amount of water.

Aqueous solutions containing 0.1–28.5% of Carbowax 1540 were sprayed on the leaves and stems of succulent, greenhouse-grown tomato plants. Those treated with the 1% solution were not injured, but others sprayed with solutions containing 9% or more of the wax developed necrotic areas in the leaves during a 10-day period following treatment. In additional experiments, kidney bean and soybean plants were sprayed with aqueous solutions containing 1–15% by weight of Carbowax 1500. The bean plants showed no toxic effects following treatment with solutions containing up to 5% wax. Primary leaves of those treated with solutions containing 10 and 15% wax became yellowish; but in general, growth and fruit production were not noticeably affected. Soybean

TABLE 1

MAXIMUM CONCENTRATIONS OF GROWTH-REGULATING SUBSTANCES DISSOLVABLE IN WATER CONTAINING 0.5% BY WEIGHT OF CARBOWAX 1500 TO MAKE SOLUTIONS TEMPORARILY STABLE AS COMPARED WITH THOSE WHICH CAN BE DISSOLVED TO MAKE STABLE SOLUTIONS. SOLUTIONS PREPARED BY DISSOLVING THE COMPOUND IN A KNOWN AMOUNT OF WAX, THEN ADDING THE WAX SOLUTION TO A KNOWN VOLUME OF WATER AT 28°–29° C.

SUBSTANCE	MAXIMUM CONCENTRATION OF SOLUTION STABLE	
	24 hours (p.p.m.)	48 hours or longer (p.p.m.)
β-indoleacetic acid	1250–1500	750–1000
Naphthalene acetamide	250– 500	125–250
2, 4-dichlorophenoxyacetic acid	1250–1500	750–1000
β-naphthoxyacetic acid	500– 750	250– 500

plants developed necrotic areas in the leaves when treated with solutions containing 5% or more of Carbowax. Potato, tomato, kidney bean, soybean, *Datura*, and a number of weeds showed no evidence of injury when sprayed with a 0.5% aqueous solution of Carbowax 1500.

RELATIVE EFFECTIVENESS OF CARBOWAX COMPARED WITH WATER AND LANOLIN EMULSIONS AS CARRIERS.—Marglobe tomato plants approximately 15 inches tall were selected for size and uniformity and divided at random into groups of six plants each. Plants of each group were sprayed with the respective treatments so as to compare epinastic responses that resulted from equivalent amounts of 2,4-dichlorophenoxyacetic acid applied in (*a*) a water solution, (*b*) a water solution to which Carbowax 1500 was added, and (*c*) a lanolin emulsion. During the following week, plants treated with the lanolin emulsion showed less epinastic response than did those treated with an equivalent amount of the acid in the wax solution (fig. 1*A*, *B*). Similar differences were observed between plants treated with the acid carried in wax solutions and others treated with the acid carried in water. These results corroborate those of ZIMMERMAN and HITCHCOCK (2).

Responses from the application of growth-regulating substance carried in water only were compared with those observed when an equal amount of the substance was carried in an aqueous solution containing 1% of Carbowax 1500. The 0.02% aqueous solution was prepared by dissolving 2,4-dichlorophenoxyacetic acid in a small amount of ethyl alcohol and adding the alcoholic solution to water. The final concentration of alcohol did not exceed 0.2%. The other solution (containing 0.02% of the acid) was prepared by dissolving the acid directly in the melted Carbowax, then adding this solution to the required amount of water to obtain a final wax concentration of 1.0%. In treating kidney bean plants, 0.05 ml. of the respective solutions was placed on the upper surface of the blade of one of the pair of primary leaves of each plant at a point on the midrib approximately $\frac{1}{4}$ inch from the base of the blade (fig. 2*A*). This amount of solution contained 10 γ of the acid, and in the case of the Carbowax solutions, 500 γ of wax. The droplet was so small that it readily adhered to the surface of the leaf. The plants used were selected from a large number and divided into groups of ten, each receiving a single treatment. Those treated with 10 γ of the acid carried in a Carbowax solution developed gall tissue which extended the full length of the first internodes, the hypocotyls became thickened, and the extension of stems and expansion of new leaves above the first internode were greatly inhibited. Galls developed in the limited amount of stem tissue above the second node in the case of many of the plants (fig. 3). In contrast, the hypocotyls and first internodes of the plants treated with an equal amount of growth-regulating substance carried in water alone showed no appreciable response, and the weight of these parts was less than that of comparable parts of plants treated with the acid solutions containing Carbowax, the difference being significant by odds of 1:100 (table 2). The growth of leaf and stem tissues above the second node was inhibited to a greater extent following treatment with acid dissolved in the Carbowax solutions than by treatment with the same amount of acid dissolved in water.

In a similar experiment, kidney bean seedlings approximately 3 inches tall

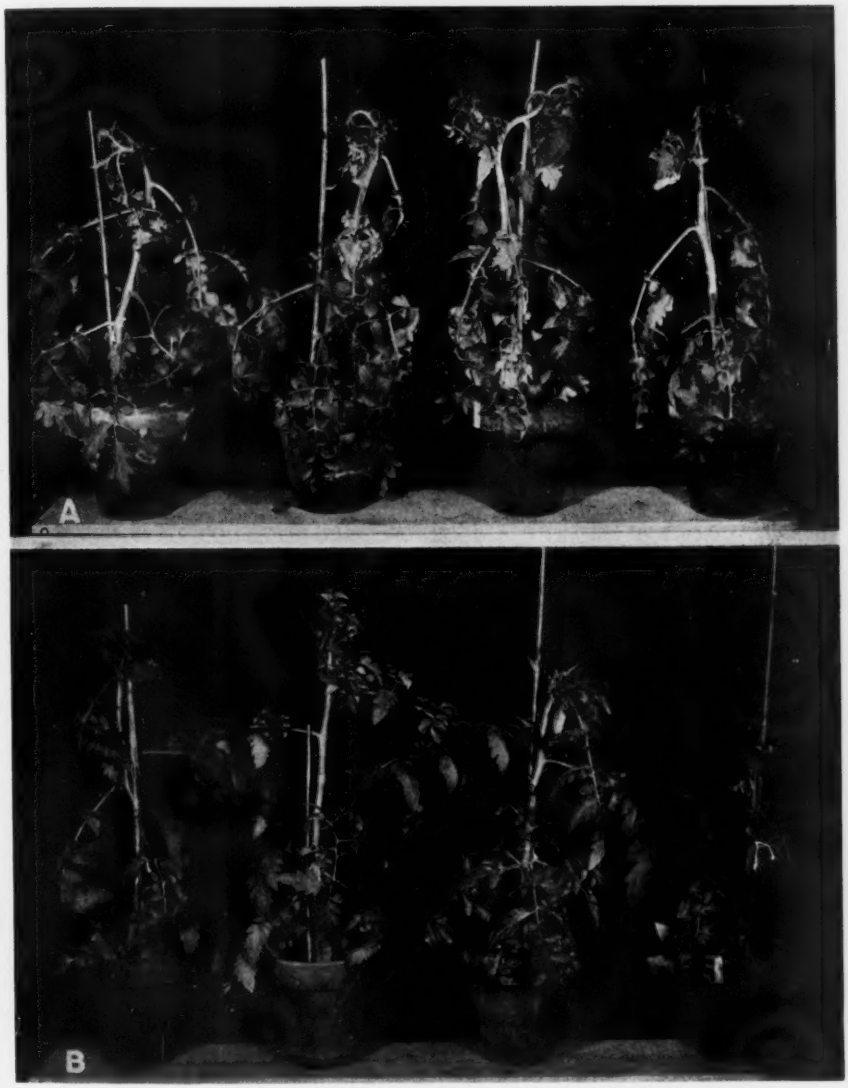

FIG. 1.—Tomato plants sprayed with: *A*, aqueous solution (75 p.p.m.) of 2,4-dichlorophenoxyacetic acid and 0.5% Carbowax 1500; *B*, lanolin emulsion containing 75 p.p.m. of the acid. Seven days after treatment.

were selected for size and uniformity. They were treated in groups of six plants each. One γ of 2,4-dichlorophenoxyacetic acid was applied to a single primary leaf of each plant in the first group by placing exactly 0.02 ml. of solutions containing 0.5% by weight of Carbowax 1500 on the upper surface of the leaf, as previously described. Plants of the second and third groups were treated in a similar manner, but with solutions containing 2.0 and 4.0 γ of the acid, respectively. Plants of the fourth, fifth, and sixth groups were also treated with 1.0, 2.0, and 4.0 γ of 2,4-dichlorophenoxyacetic, respectively, but in these latter treatments the acid was carried in water containing 0.2% by volume of ethyl alcohol in which the acid was first dissolved, but no Carbowax. During the following 2 days those treated with solutions containing Carbowax and 1 γ showed stem curvatures and slight epinasty, and those treated with 2 and 4 γ of the acid carried in wax showed marked stem curvatures and epinasty. Where no Carbowax was present, only those treated with 4 γ of the acid exhibited a noticeable response, and even this was clearly less than that resulting when an equal amount of acid was applied together with the wax (fig. 4). Terminal bud growth was inhibited to a greater extent when the acid was applied together with Carbowax than when applied in a dilute alcoholic solution. The average fresh weight of growth above the primary leaves of plants treated with 1 γ of acid and 250 γ of Carbowax was 570 mg. on the seventh day following treatment; that of other plants treated with an equal amount of acid but without the addition of wax, 1340 mg. Application of 4 γ of 2,4-dichlorophenoxyacetic acid together with 250 γ of wax resulted in greatly reduced terminal bud

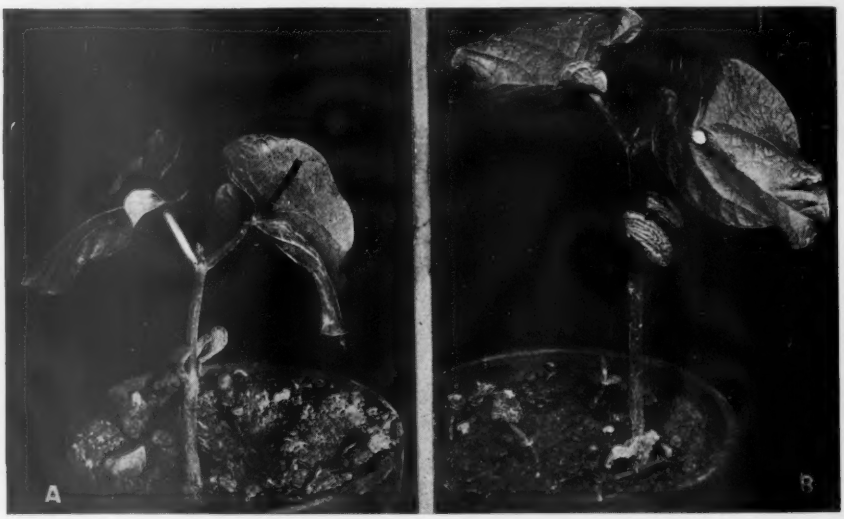

FIG. 2.—Methods of applying growth-regulating substances, using: *A*, 0.02 ml. of aqueous solution containing substance and Carbowax (arrow indicates position along midrib on primary leaf where application was made); *B*, pellet of Carbowax in which growth substance was dissolved.

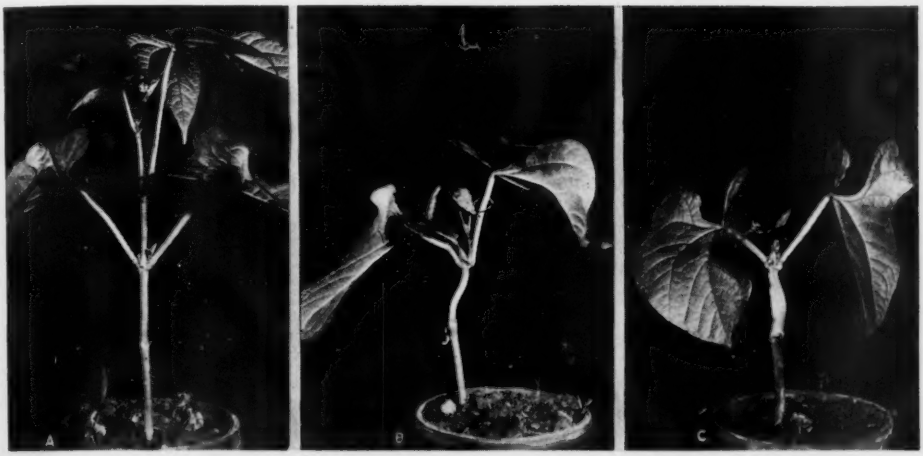

FIG. 3.—Effects of treating kidney bean seedlings with: *A*, 500 γ of Carbowax 1500 in aqueous solution; *B*, 10 γ of 2,4-dichlorophenoxyacetic acid in dilute alcoholic solution; *C*, 10 γ of acid together with 500 γ of Carbowax in aqueous solution. Eleven days after treatment.

FIG. 4.—Effects of 4 γ of 2,4-dichlorophenoxyacetic acid on growth of individual plants (each treated as illustrated in fig. 2*A*): *A*, acid dissolved in 0.2% aqueous solution of alcohol; *B*, acid dissolved in aqueous solution containing 0.5% Carbowax 1500. Fourteen days after treatment.

growth, the average weight of the buds being 230 mg. as contrasted with 660 mg. for the terminal buds of plants treated with an equal amount of acid to which no wax was added.

Additional experiments were conducted to determine the efficiency of Carbowax alone as a carrier for growth-regulating substances, as compared with Carbowax to which water had been added. Kidney bean seedlings that had been selected for size and uniformity were divided into groups. Plants of one group were treated individually with portions of no. 1500 approximately the size of a wheat grain and containing 1% of 2,4-dichlorophenoxyacetic acid. The wax was placed on the upper surface of one of the primary leaves of each plant and along the midrib at a distance of approximately $\frac{1}{4}$ inch from the petiole attachment (fig. 2*B*). Plants of the second and third groups were treated in a similar manner with wax containing 0.1 and 0.02% of the acid, respectively. For comparison with the latter treatment, one drop of a 0.5% aqueous solution of Carbowax 1500 containing 0.02% of the acid was placed on each leaf in a similar manner. This volume of solution contained approximately the same amount of acid as did the portion of 0.02% wax-acid mixture used. Other plants were treated with the wax alone, and others were left untreated as controls.

TABLE 2

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON GROWTH OF BEAN PLANTS WHEN APPLIED TO THE PRIMARY LEAVES IN AQUEOUS SOLUTIONS WITH AND WITHOUT CARBOWAX. MEASUREMENTS 12 DAYS AFTER TREATMENT

WEIGHT APPLIED		AVERAGE WET WEIGHT (GM.)		
Acids (γ)	Wax (mg.)	Hypocotyls	First internodes	Tops*
10.....	0.5	1.81	1.05	0.74
10.....	0.0	1.42	0.68	1.34
0.....	0.5	1.36	0.57	3.50

* All stem and leaf tissues above primary leaves.

One per cent of the acid in Carbowax resulted in the death of tissues of the leaf blades, while the stems showed marked growth responses (fig. 5). Bud growth was greatly inhibited. Similar—but much less intensive—responses resulted from treatment with wax containing 0.1% of the acid. A concentration of 0.02% acid in wax resulted in relatively slight growth responses, but the plants produced flower buds. How-

FIG. 5.—Effects of approximately 100 mg. of 1% mixture of 2,4-dichlorophenoxyacetic acid in Carbowax applied as solid (fig. 2*B*) to lefthand leaf only. Terminal bud suppressed; large tumor extends entire length of first internode and hypocotyl (effects often extend into roots also). Fourteen days after treatment.

ever, the same concentration of acid (0.02%) applied in a 0.5% aqueous solution of wax resulted in marked growth responses and complete inhibition of terminal bud growth. Growth of lateral buds was much reduced, but a few small flower buds developed during a 2-week interval following treatment. Similar effects were also noted in other experiments in which different amounts of 2,4-dichlorophenoxyacetic acid in a 0.5% aqueous solution of Carbowax 1500 was added in 50-ml. aliquots to the soil in which seedling bean plants were growing (fig. 6).

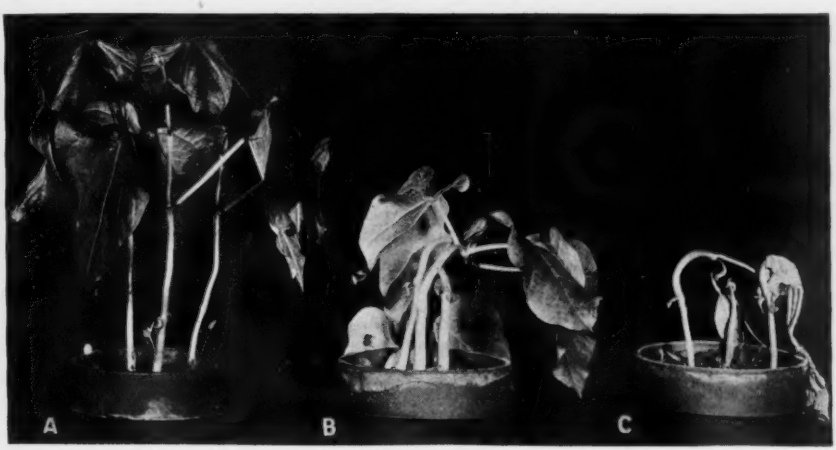

FIG. 6.—Effects of 2,4-dichlorophenoxyacetic acid in aqueous solution containing 0.5% Carbowax on kidney beans applied to soil in which the plants were growing. *A*, control; *B*, 5 mg. and *C*, 50 mg. of acid applied to each 4-inch pot. Fourteen days after treatment.

To observe the effect of growth-regulating substances carried in Carbowax on the formation of abscission layers, debladed petioles of *Coleus* were treated on the cut surface with no. 1500 wax containing 1% by weight of β-indoleacetic acid. Other comparable petioles were treated with the wax alone, while others were left untreated for comparison. All petioles treated with the wax alone, and also those untreated, abscised within 10 days following treatment. Those treated with the acid-wax solution were killed back to within 3–4 mm. of the stem, but these basal ends remained attached to the stems during a period of 10 days following treatment, after which the experiment was discontinued.

In a subsequent experiment, eight uniform plants of *Coleus* were selected. The blades were detached at the extreme outer end of the petioles from approximately half the leaves of each plant. The plants were then divided into two groups of equal numbers. One group was sprayed on the first and third days of the experiment with a 0.5% aqueous solution of Carbowax 1500 that contained 50 p.p.m. of α-naphthaleneacetic acid. The remaining group was left untreated. In the respective treatments, 13.6, 0.0, 0.0, and 3.6% of the debladed petioles abscised during the 8 days immediately following treatment, in contrast to 82.8, 75.8, 100.0, and 97.8% in the case of untreated plants.

Discussion

The hygroscopic nature of Carbowax 1500 and 1540, together with the solubility of growth-regulating substances in them and their capacity as wetting agents, is advantageous in relation to their use as carriers. Employed in this manner, they effect the accumulation and retention of moisture on the surface of the plant, and as a result the growth-regulating substance tends to remain in solution and in close contact with the surface over a relatively long period. Because of these characteristics, polyethylene glycols may be useful as carriers for plant fungicides or insecticides, although no experiments on this problem were undertaken in connection with the present work. Since Carbowax compounds are soluble in water, relatively concentrated stock solutions of growth-regulating substances and wax can be prepared and the desired amount dissolved in water as needed. A wide range of concentrations of different growth substances can be prepared in this manner.

2,4-Dichlorophenoxyacetic acid was extremely toxic when applied in relatively high concentrations together with Carbowax to the stems, leaves, or roots of plants tested. When applied to one part of the plant, such as a leaf, the influence of such solutions was transmitted throughout seedling plants both upward and downward, and at certain concentrations the plants were killed. The results reported here, and similar ones from other experiments, indicate that the effectiveness of 2,4-dichlorophenoxyacetic acid and a number of related compounds may be increased by the addition of an appropriate carrier and wetting agent. Since these compounds, or some derivatives from them, apparently travel for long distances through certain plants (1), they may prove highly effective as herbicides, even when applied in low concentration. It was also noted that certain of the weeds, particularly grasses, which were growing in soil treated with solutions of 2,4-dichlorophenoxyacetic acid were differentially affected. This may be of importance in connection with the differential killing of weeds.

Although Carbowax was used in these experiments, the use of other carriers or wetting agents might also increase the effectiveness of certain growth-regulating substances in this respect.

In testing a great number of solutions containing different combinations of Carbowax, other carriers, and various growth-regulating substances for their effectiveness in producing growth responses, it was desirable to use a simple method in which the results could be quantitatively expressed. Such a method consisted in the use of kidney bean plants grown under greenhouse conditions. They are relatively sensitive to the presence of most growth-regulating substances. When solutions or mixtures containing the compounds were applied in appropriate amounts to the upper surface of the primary leaves of such plants, the resultant responses are readily observed and measured in terms of the difference between the weight of the parts most readily affected (such as the terminal bud, first internode, or hypocotyl) and the weight of comparable parts of control plants. Subsequent histological or cytological studies likewise may be related to more precisely determined dosages. In standardizing such tests it was found convenient to apply quantitatively 0.02 ml. of the solution to be tested to the upper surface of the leaf along the midrib and near the petiole attachment. By such means it was possible to treat a relatively large number of uniform plants with little effort and to compare at any

one time the relative effectiveness of a number of solutions containing different compounds or various proportions of the same compound.

Summary

1. Addition of Carbowax compounds to aqueous solutions of 2,4-dichlorophenoxyacetic acid increased the effectiveness of the acid in bringing about growth responses and form changes in kidney bean plants as measured on a quantitative basis.

2. Marked form changes occurred in roots, hypocotyl, first internode, and terminal and lateral buds as the result of application of 4 γ of the acid to one primary leaf of seedling bean plants. Application of 1 γ of the acid in a like manner resulted in greatly inhibited bud growth.

3. A 0.5% solution of Carbowax 1500 was found to be nontoxic to several kinds of crop plants.

4. Application of relatively high concentrations of 2,4-dichlorophenoxyacetic acid in solution with Carbowax killed the bean plants when applied either to the soil or above-ground portions. The possibility of using this compound, and various others together with Carbowax, as selective herbicides is suggested.

5. A relatively simple quantitative method of measuring the effectiveness of growth-regulating compounds in bringing about growth responses is described. It is based on the application of exact amounts of the substance to a bean leaf and the subsequent measurement of the growth changes in the stem and buds.

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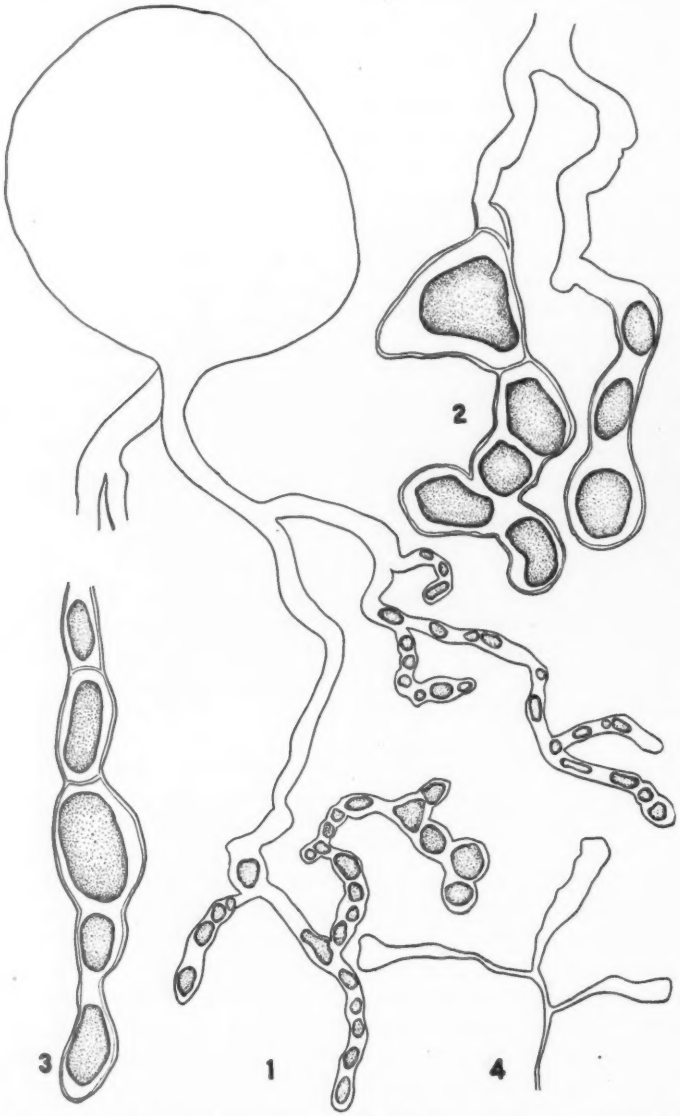
SPECIES OF BOTRYDIUM FROM NORTHERN INDIA

M. S. RANDHAWA

The genus *Botrydium* is well represented in India. *B. granulatum* is more or less universally distributed, appearing on moist mud in the middle of the monsoon period. It may be encountered up to the month of March in shaded localities on the sides of ponds, lakes, and banks of freshwater streams and rivers. A club-shaped variety of *B. granulatum* was recorded by IYENGAR (2) from Nandhi Hills in Mysore in December, 1924, and was described as a new variety (var. *clavaeformis*) by the writer (4) from Fyzabad in February, 1940. *B. tuberosum* Iyengar, a dwarf species with tuber-like solitary terminal cysts, was described by IYENGAR from Madras in 1924 and was recorded by the present writer from Fyzabad in northern India in February, 1939 (3). A variety of *B. tuberosum* was described by RAO (5) from Lucknow in 1935 and named by him as var. *intermedium*, for he considered it a linking form between *B.*

granulatum and *B. tuberosum*. *B. divisum* Iyengar, a form with divided subaerial portion, was described by IYENGAR from Calcutta in January, 1923, and a form resembling this alga was described by the writer from Fyzabad in August, 1937.

The object of this note is to describe a new variety of *B. granulatum* from Rae Bareli, to record the presence of *B. divisum* from Rae Bareli, and to describe cyst formation in *B. granulatum* var. *clavaeformis* Randh.



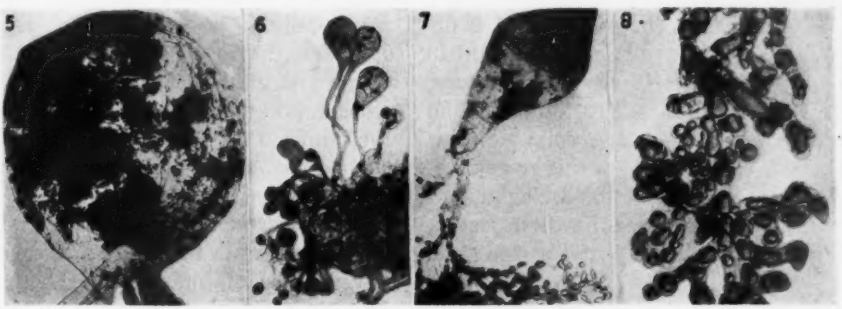
FIGS. 1–4.—Figs. 1–3, *Botrydium granulatum* var. *polyrhiza* var. nov.: Fig. 1, cyst formation. Figs. 2,3, rhizoids enlarged to show types of cysts. Fig. 4, *B. divisum;* trilobed subaerial thallus.

1. *Botrydium granulatum* (L.) Grev. var. *polyrhiza* var. nov.—This variety was collected from semidried mud heaped along the sides of an embankment of a reservoir at the village of Tiloi in Rae Bareli district of the United Provinces on January 28, 1943. The above-ground vesicular portion is globose (fig. 5) or slightly pear-shaped (fig. 1), as in typical specimens of *B. granulatum*, and is 1–1.80 mm. in diameter.

In the structure of its underground rhizoidal portion this alga differs from all the recorded varieties. While in other varieties of *B. granulatum*, or even in other species of *Botrydium*, there is an intermediate cylindrical portion connecting the lower branched rhizoidal portion with the above-ground vesicle, in this form there is no such portion, and two to five rhizoidal branches arise from the very base of the above-ground vesicle (figs. 1, 5). This type of habit for *B. granulatum* has not been recorded so far and justifies the creation of a new variety, which has been named as var. *polyrhiza*.

Many of the plants were in an advanced state of cyst formation, which in this alga differs from that of typical specimens of *B. granulatum* in the wider separation of cysts from one another and in their shape (figs. 1–3). In most cases terminal cysts are bigger than intercalary ones. Occasionally such terminal cysts are rounded, as in *B. tuberosum* var. *intermedium* described by RAO (fig. 2). The cysts are 24–72 μ broad.

2. *Botrydium tuberosum* Iyengar.—The above-ground vesicle is globose to pear-shaped and 96–200 μ in diameter. The rhizoidal portion bears solitary globose to terminal cysts at the ends of the rhizoids (fig. 6). It was found growing gregariously on the banks of a lake at the village of Shamaspore, district Rae Bareli, on January 11, 1943. It covered a large area, particularly on patches manured by the droppings of wild ducks and geese.



FIGS. 5–8.—Fig. 5, *Botrydium granulatum* var. *polyrhiza* showing five main rhizoidal branches arising from subaerial thallus. Fig. 6, *B. tuberosum*, cyst formation. Figs. 7, 8, *B. granulatum* var. *clavaeformis:* Fig. 7, club-shaped subaerial vesicle and cyst formation in rhizoids; fig. 8, detail of cyst formation.

The alga described by RAO as *B. tuberosum* var. *intermedium* has been called var. *intermedium* under the impression that it is a linking form between *B. tuberosum* and *B. granulatum*. So far as size of the above-ground vesicle and method of formation of cysts—as well as their shape—are concerned, this form resembles *B. granulatum*, and particularly the multi-rhizoidal variety just described, rather than *B. tuberosum*. *B. tuberosum* is a dwarf variety which seldom exceeds 200 μ in diameter in northern India, although IYENGAR has given 500 μ as the maximum size for samples collected from Madras in south India.

RAO has given the size of the alga described by him as 0.5—0.75 mm. Apart from size, the presence of serial intercalary cysts definitely connects the Lucknow alga with *B. granulatum* rather than with *B. tuberosum*—which has only solitary cysts at the terminal ends of rhizoids. Apparently var. *intermedium* should be described as a variety of *B. granulatum* rather than of *B. tuberosum*, from which it differs in size of above-ground vesicle, in the size, shape, and position of cysts, and in the method of cyst formation.

3. Cyst formation in *B. granulatum* var. *clavaeformis*.—This elongated, club-shaped variety of *B. granulatum* was described by the writer from Fyzabad in 1940. However, only vegetative features were described, as no cyst formation had occurred in the specimens collected. This alga was again found growing gregariously in big patches from the drying mud of a pond at Maharajganj in Rae Bareli, January, 1943, and plenty of ripe cysts were seen.

Above-ground vesicles are elongated, club-shaped (fig. 7), and 350–900 μ in diameter. Cysts are formed serially in rhizoids, usually in single rows; although multiserial, *Palmella*-like patches are also seen. Such *Palmella*-like clusters have not been reported before. Cysts are irregularly elliptic, ovoid, triangular or irregular in outline (fig. 8), and 18–60 μ in diameter.

A few trilobed specimens which resemble *B. divisum* were found mixed with *B. granulatum* var. *clavaeformis* at Maharajganj. These resembled the samples described from Fyzabad by the writer. In this case the lobes are widely separated (fig. 4) and 120–210 μ in diameter at the broadest point. Tendency toward branching is seen in the young plants of *B. granulatum* var. *clavaeformis*. This fact, coupled with the occurrence of sporadic specimens of this lobed form among the plants of the club-shaped variety of *B. granulatum*, raises the presumption that it may be merely its habitat form.

In 1939 I (3) described squarish cysts from the lower part of the thallus of some plants of *B. divisum*. This is a very unusual position for cysts in a species of *Botrydium*, for in all other species they are formed in the rhizoids. It may be added, however, that the part of the alga from which these cysts were described had become buried in soil, as was evident from dust particles clinging to the sides. In *B. granulatum* as well, cysts may be sometimes seen in the vesicular portion. Describing cyst formation in *B. granulatum*, SMITH (6) observes that "hypnospores [cysts] may also be found in vesicular portion of the plant body, either from multinucleate or from uninucleate aplanospores." In this case also, the cause appears to be the burying of the above-ground thallus under soil, and thus the production of the same physiological conditions under which cyst formation is induced in subterranean rhizoids.

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CURRENT LITERATURE

Pathology in Forest Practice. By D. W. BAXTER. New York: Wiley & Sons, 1943. Pp. 618. Figs. 232. $5.50.

The problems of pathology in forest practice are discussed in the aspect in which they present themselves to the forester in the nursery, the plantation, the forest, the park, and in industry; consequently, little attention is paid to the small details of taxonomy, mycology, and etiology. Stress is placed on a working knowledge of the conditions which favor the development of various diseases and on the practical measures which can be taken to reduce their incidence or severity.

After a discussion of the nature of a plant disease, the causal fungi are briefly described. Emphasis is placed on the higher Basidiomycetes because they include the principal wood-destroying fungi. Various methods are given of determining loss and of making an appraisal of damage. Particular attention is given to critical relationships, such as (*a*) those of site and of cultural practice to the incidence of disease in the nursery, in plantations, and in the mature forest; (*b*) those of fungi and of certain other plant pests to disease in the mature forest; and (*c*) those of site and of care given shade and park trees to disease. Forest products are considered from the standpoints of decays, discoloration, stains, molds, and other defects.

This book is particularly timely, since during the war devastating inroads are being made on the nation's timber supply and because after the war many returned soldiers and war workers will find employment in extensive reforestation. BAXTER has assembled the information for those planning and directing the forestry programs so that costly and time-consuming errors may be avoided. He has attacked a difficult problem in a masterful and authoritative way.

The printing is excellent, both of the text and of the illustrations. A large number of literature citations appear at the end of each chapter. There is an excellent index.

Among technical books on forestry, this one will appeal to practical men. It is indispensable for anyone seeking to understand the conditions which favor tree damage by disease and with this information either to provide protection or to reduce the severity of the injury.—A. J. RIKER.

Illustrated Flora of the Pacific States—Washington, Oregon, and California: Vol. II. By LEROY ABRAMS. Stanford University: Stanford University Press, 1944. Pp. viii+635. Figs. 1663 (nos. 1300–2962).

The first volume of this flora appeared in 1923. It was announced at that time as the first of three volumes. The plan has been changed since to provide for four volumes. Volume I presented the Ophioglossaceae to the Aristolochiaceae. The present volume includes the Polygonaceae to the Krameriaceae. It has certain portions contributed by specialists and embodies the assistance of many collaborators, but in the main represents the indefatigable labor and profound scholarship of the author himself. The "Britton and Brown" style used in volume I, of illustrating each species singly, has been replaced by another, that of grouping several or many figures upon one page. The illustrations are excellent.

A large number of varieties and "subspecies" are included. In most cases no key is given for these. This seems unfortunate. Moreover, the retention side by side of large numbers of varieties and subspecies will doubtless help hasten the day when taxonomists will courageously face the vexatious problem of just how far to permit or condone the use of the *subspecies* category. For the present, students and laymen using this work and seeing now a variety and now a subspecies, might well attribute to its author a divining insight into nature which he surely would be the first to disavow.

Numerous species are treated in small type and interpolated here and there without an assigned number among the species of their respective genera. In many such cases (for example, *Heuchera Merriamii*, p. 379) it is not clear as to whether doubt is thus implied regarding their validity or whether they are actually intimated to be synonymous. That they are hardly last-minute inclusions is to be inferred from the long list (p. 629) of new entities "described in 1938–1943" and summarized in the Appendix. Curiously, too, various assumedly new combinations (for example, *Saxifraga adscendens* subsp. *oregonensis*, p. 359; *Astragalus Fremontii* subsp. *eremicus*, p. 597) are published, but with no indication that they are new. In the main, however, the text represents a high standard of attainment and more than fulfils the high expectations of the many botanists who have for years awaited its appearance.—E. E. SHERFF.

The Chemistry of Natural Coloring Matters. By FRITZ MAYER; translated and revised by A. H. COOK. New York: Reinhold Publishing Corporation, 1943. Pp. 354.

This is one of the series of monographs published by the American Chemical Society. It is an excellent review of the advances made during the last 15 years in the chemistry of the pigments found occurring naturally in plants and animals. The literature has been summarized up to the summer of 1941.

The organization of the work is based upon the chemical nature of the pigments considered. The first chapter considers the polyene pigments commonly known as carotenoids. Almost fifty of these are now known and described in chemical terms, although about one-third of them yet lack acceptable

formulas. A very brief chapter discusses curcumin, under the heading of diaroylmethane compounds. It is the only pigment belonging to this group. Curcumin is used by some as a dye.

The third chapter presents the chemistry of carbocyclic compounds. Many of these are related to benzoquinone, many others to naphthoquinone, and still others to anthracene. Some of the anthracene pigments occur in insects. A few pigments are related to phenanthrene; in all of them the rings are composed only of carbon atoms. The last two chapters consider the heterocyclic pigments, one chapter devoted to the compounds containing oxygen in the rings and one chapter discussing those containing heterocyclic nitrogen atoms in the rings.

The plant physiologist will find the volume very valuable from the chemical viewpoint. The carotenoids, anthocyanins, flavones, etc., are so fully treated that the monograph leaves little to be desired on the chemistry of the pigments. The real gap in our knowledge, the place where much more research must be done and where little information is yet available, pertains to the biosynthesis of these pigments in living plants and animals—mainly in plants. It is relatively easy to determine the relationships of the pigments, but their origin is still shrouded with ignorance. For instance, when it was found that the carotenoids were constructed from isoprene units, one might have supposed that the biosynthesis would soon be made plain. It is not too much to hope, however, that the biochemists will ultimately solve the problems of pigment manufacture within the cell. When one views the enormous advances that have been made in little over a decade with the chemistry of these compounds, it is a challenge to carry the work back into the originating physiological processes.

This is an excellent monograph, worthy of careful study by anyone who wants to grasp the chemical nature of the natural pigments.—C. A. SHULL.

Marine Algae of the Monterey Peninsula. By GILBERT M. SMITH. Stanford University: Stanford University Press, 1944. Pp. vii+622. Illustrated. $6.00.

This volume is a comprehensive treatise containing the great majority of the species occurring along the western coast of the United States, in spite of restriction of the species covered to the Monterey Peninsula. It is estimated that the marine flora of this peninsula comprises at least 80% of the species found between Puget Sound and southern California. The flora of the peninsula is far richer than that of any other region of the west coast, and the area is noteworthy also in that it is the type locality of about one-fourth of the species of the west coast.

The descriptions of the species are based upon specimens collected from the Monterey Peninsula and are not simply a re-phrasing of previous descriptions. An illustration is given for nearly every one of the almost 400 species described. These drawings bring out the general characteristics of the plants, and in some cases details of vegetative or reproductive structures are also shown. The illustrations are an important feature of the book and help greatly in identification of the species. Comprehensive keys, based almost wholly upon external form and internal vegetative structure, are given for the Chlorophyta, Phaeophyta, and Rhodophyta treated. These keys have been revised following repeated use by students of the author.

The book is an important contribution to the literature dealing with marine algae and will be especially valuable for those interested in the marine flora of the west coast of the United States.—J. M. BEAL.

GENERAL INDEX

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